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(57) Abstract

A novel CC chemokine, TSG–5, TSG–5vl or TSG–5v2, is described, along with methods of making and using TSG–5, TSG–5vl or TSG–5v2 and related nucleic acids and antibodies thereto. The invention also relates to methods of identifying agents which modulate the activity of TSG–5, TSG–5vl or TSG–5v2 and/or its ligands.

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TITLE: TSG–5: A TNF–INDUCIBLE GENE

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This application is related to U.S. Provisional Application 60/102,904, filed October 2, 1998, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to a novel CC chemokine, TSG-5 and novel TSG-5v1 and TSG-5v2.

BACKGROUND OF THE INVENTION

1. Cytokines

Cytokines are a growing family of regulatory proteins which control the growth, development, and bioactivities of hematopoietic and immune cells. Cells targeted by cytokines include bone marrow, peripheral blood, fetal liver, and other lymphoid or hematopoietic organs. Chemokines, a subset of cytokines, are one of three types of factors which induce chemotaxis.

2. Chemokines

Chemokines (also known as intercrines and SIS cytokines) belong to the pro-inflammatory class of cytokines. Chemokines are basic, heparin-binding proteins that have proinflammatory and reparative activities. They are distinguishable from other proinflammatory cytokines with reparative activities (*e.g.*, interleukin-1 and platelet-derived growth factor) by their characteristic conserved single open reading frame, signal sequences in the N-terminal region, AT-rich sequences in their 3'-untranslated regions, and rapidly inducible mRNA expression (Charo *et al.*, 1998 U.S. Patent No. 5,707,815). Typically, chemokines range in molecular mass from 8-10 kilodaltons (kD) and are 70-100 amino acids in length. In humans, chemokines are the products of distinct genes

clustered on chromosomes 4 and 17. For additional details, see Thomson, THE CYTOKINE HANDBOOK, Second Edition (Academic Press, NY; 1994). Chemokines are induced by primary proinflammatory mediators, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF).

Chemokines play a major role in inflammatory responses and affect hematopoiesis both negatively and positively. Chemokine-mediated diseases include: psoriasis, rheumatic diseases, osteoarthritis, gout, arthritic diseases and other inflammatory diseases, idiopathic pulmonary fibrosis, sepsis (Fujishima *et al.*, 1996 Intensive Care Med. 22: 1169-1175) and certain malignancies. Some chemokines inhibit cell proliferation, cell cycling and are myelosuppressive (see Beutler *et al.*, 1998 U.S. Patent No. 5,770,402). Such chemokines protect progenitor cells from the effects of chemotherapy and radiation therapy (see Daly *et al.*, 1998). Chemokines may modulate calcium flux in certain tissues and may be useful in the treatment of T cell mediated diseases (*e.g.*, rheumatoid arthritis, multiple sclerosis, inflammatory bowel diseases, allergy, contact hypersensitivity, psoriasis, systemic lupus erythematosus, *etc.*).

The main activity of chemokines is chemotaxis. Chemokines attract or activate a variety of cell types including leukocytes, endothelial cells and fibroblasts. One chemotactic activity of chemokines is leukocyte recruitment to promote extravasation of inflammatory cells to the site of inflammation or tissue injury. Although several chemokines are expressed by many tissues, leukocytes comprise one of the main producers of chemokines. However, unlike classic leukocyte chemoattractants, which possess little specificity, members of the chemokine family induce recruitment of well-defined leukocyte subsets. For example, the CC chemokine RANTES specifically activates CC chemokine-activated killer cells (CHAK) (Maghazachi *et al.*, 1996 Eur. J. Immunol. 26: 315-319). CC chemokines can also directly increase inflammation and can induce cell proliferation and activation (Elsner *et al.*, 1996 Eur. J. Immunol. 26: 1919-1925; Maghazachi *et al.*, 1996).

A. Classes of Chemokines

All chemokines have four cysteine residues, which form two disulfide bridges which are a higher conserved motif in their primary amino acid structure. Chemokines are classified based upon the amino terminal pair of these cysteines. The four classes of chemokines are the "CC", the "CXC", the "CX_3C" and the "C" chemokines, wherein X can be any amino acid.

The CC chemokines are chemotactic factors for monocytes, basophils and eosinophils. The CXC chemokines are generally attractants for neutrophils but not mononuclear cells. The CX_3C chemokines, of which only one is known, is chemotactic for T cells, monocytes and neutrophils. The C chemokines also only have one identified member, lymphotactin, which is chemotactic for $CD8^+$ T cells. The two most described groups of chemokines are the CC and CXC forms.

The genes encoding CXC chemokines (also known as α chemokines) are located on chromosome 4 and include: interleukin-1 (IL-8), β-thromboglobulin (βTG), platelet factor 4 (PF-4), interferon inducible protein 10 gamma (IP-10γ), growth stimulating factor (GRO, which includes GROα, GROβ, and GROγ forms; GRO is also referred to in the medical literature as melanoma growth stimulating factor, MGSD), and murine macrophage inhibitory protein-2 (MIP-2) (see Daly *et al.*, 1998 U.S. Patent No. 5,789,539).

In the CC chemokine family, also referred to as the β subfamily, the amino terminal pair of cysteine residues are located adjacent to each other in a C-C linear arrangement. CC chemokine genes are located on human chromosome 17 (mouse chromosome 11). Homology in the CC subfamily ranges from 28-48% intraspecies and 25-55% interspecies. Exemplary members include the protein I-309, ACT-2, RANTES, JE factor (murine homologue of MCP-1), macrophage inhibitory protein-1α and β forms (MIP-1α and MIP1-β), eotaxin and TCA-3 (Charo *et al.*, 1998 U.S. Patent No. 5,707,815; Godiska *et al.*, 1997 U.S. Patent No. 5,688,927; Wilde *et al.*, 1997 U.S. Patent No. 5,602,008; Sanz *et al.*, 1998 J. Immunol. 160: 3569-3576).

CC chemokines are associated with inflammation, and certain CC chemokines have been utilized in medical imaging to image sites of infection, inflammation or

localization of CC chemokines (See, for example, Lyle *et al.*, 1997 U.S. Patent No. 5,605,671 and related applications).

B. Chemokine Receptors

Chemokine-chemokine receptor interactions result in many cellular activities involved with immune responses. Chemokines act as chemoattractants for human monocytes *in vitro;* agonists of cytosolic free calcium and respiratory bursts in monocytes; inducers of monocyte-mediated tumoristatic activity and tumoricidal activity; mediators of inflamed tissue infiltration by monocytes; mediators of monocyte infiltration into virus infected tissue (Hofmann *et al.*, 1997 J. Leukocyte Biol. 61: 408-414); and inducers of myositis related inflammation (Adams *et al.*, 1997 Proc. Assoc. Am. Phys. 109: 275-285). Chemokines bind to chemokine receptors expressed on the surface of targeted cells. The CC chemokine receptors of MIP-1α and RANTES have been characterized as members of the G-protein-linked receptors containing seven transmembrane-spanning domains (Loetscher *et al.*, 1996 J. Exp. Med. 184: 569-577). Certain CC chemokine receptors (*e.g.*, CCR2 and CCR5) have been identified as co-receptors involved in the infection process of HIV-1 (Kissler *et al.*, 1997 Clin. Immunol. Immunopathol. 84: 338-341; and Mackewicz *et al.*, 1997 J. Clin. Invest. 100: 921-930). For additional material, see Colowick, 1997 "Chemokine Receptors," Methods in Enzymology 288.

C. Methods of Regulating Chemokines

Chemokines are induced by proinflammatory cytokines such as TNF, IL-1 and interferon-γ (IFN-γ) (Li *et al.*, 1996 J. Invest. Dermatol. 106: 661-666). External agents, such as bacteria, can also induce production of chemokines. For example, *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* all strongly induce IL-8 and MIP-1α synthesis by macrophages (Hachicha *et al.*, 1998 J. Immunol. 106: 449-454). Chemical agents, such as rhodamine-MIP-1α, can also induce CC-chemokines (Solari *et al.*, 1997 J. Biol. Chem. 272: 9617-9620).

Agents, such as corticosteroids can down-regulate chemokine synthesis. Corticosteroids inhibit production of such chemokines as RANTES and IL-1 (Meltzer, 1997 Allergy: Eur. J. Allergy Clin. Immunol. Suppl. 52: 33-40). Monoclonal antibodies have been demonstrated to inhibit the activity of various chemokines. For example, anti-eotaxin antibodies inhibited eotaxin stimulation of eosinophil adhesion to human lung microvascular endothelial cells (Burke-Gaffney *et al.*, 1996 Biochem. Biophys. Res. Commun. 227: 35-40), and anti-MCP-1 antibodies inhibited monocyte attraction to tissues where MCP-1 was present (Peri *et al.*, 1994 J. Immunol. Methods 174: 249-157). Other known chemokine inhibitors include: Met-RANTES, viral chemokine inhibitor (vCC1), viral chemokines, antileukinate and other peptides, curcumin and other chemokine mRNA synthesis inhibitors, anti-chemokine receptor antibodies, AOP-RANTES, α-melanocyte-stimulating hormone (α-MSH), interleukin-10 (IL-10), α-methyl-D-mannoside (αMM), anti-Herpes simplex virus gD antibodies, pentamidine, bisindolylmaleimide compounds, interleukin-13 (IL-13), cholera toxin, N-(2-hydroxy-4-nitrophenyl)-N'-(2-bromophenyl)urea, other chemokines such as monocyte chemotactic protein-2 (MCP-2), and a 35 kD chemokine binding protein (vCKBP) (Elsner *et al.*, 1997 Eur. J. Immunol. 27: 2892-2898; Smith *et al.*, 1997 Virology 236: 316-327; Krathwohl *et al.*, 1997 Proc. Nat'l Acad. Sci. USA 94: 9875-9880; Hayashi *et al.*, 1997 J. Clin. Invest. 99: 2581-2587; Xu *et al.*, 1997 Exp. Hematol. 25: 413-422; Wu *et al.*, 1997 J. Exp. Med. 185: 1681-1691; Simmons *et al.*, 1997 Science 276: 276-279; Chiao *et al.*, 1997 J. Clin. Invest. 99: 1165-1172; Cassatella *et al.*, 1997 Eur. J. Immunol. 27: 111-115; Yamamoto *et al.*, 1996 Infect. & Immun. 64: 3062-3068; Van Wauwe *et al.*, 1996 Inflamm. Res. 45: 357-363; Jordan *et al.*, 1996 Br. J. Pharmacol. 117: 1245-1253; Su *et al.*, 1996 J. Virol. 70: 1277-1281; De Vries *et al.*, 1995 Int. Arch. Allergy Immunol. 106: 175-179; Maghazachi *et al.*, 1994 J. Immunol. 153: 4969-4977; White *et al.*, 1998 J. Biol. Chem. 273: 10095-10098; Proost *et al.*, 1998 J. Immunol. 160: 4034-4041; and Alcami *et al.*, 1998 J. Immunol. 160: 624-633, respectively). Most of these CC chemokine inhibitors are specific to one chemokine. However, one CC chemokine homolog encoded by the MC148 gene of the *molluscum contagiosum* virus, competitively inhibits the activity of human CC chemokines. The MC148 protein binds to several types of CC chemokine

receptors and thereby competitively inhibits the binding of the human CC chemokines to these receptors (Damon *et al.*, 1998 Proc. Nat'l Acad. Sci. 95: 6403-6407).

D. Methods of Isolating Chemokines

Several CC and CXC chemokines have been isolated using differential hybridization. For example, macrophage-derived chemokine (MDC) was isolated from a human macrophage cDNA library using monocyte- and macrophage-specific cDNA probes (Rodenburg *et al.*, 1998 J. Leukocyte Biol. 63: 606-611). Another chemokine so isolated is Mob-1 (Zhang *et al.*, 1997 Oncogene 14: 1607-1610; and Liang *et al.*, 1994 Proc. Nat'l Acad. Sci. USA 91: 12515-12519). See also Yun *et al.*, 1998 Am. J. Physiol. Heart Circ. Physiol. 274: H331-H341; and Grady *et al.*, 1997 Gastroenterology 113: 1966-1975).

E. Clinical Importance of CC Chemokines

CC chemokines are chemoattractants for monocytes, lymphocytes, eosinophils and basophils, but not neutrophils (Weber *et al.*, 1995 Int. Arch. Allergy Immunol. 107: 148-150). Therefore, chemokines serve as signal agents in immune response cascades. CC chemokines are also known to be involved in asthma (*e.g.*, eotaxin, Dairaghi *et al.*, 1997 J. Biol. Chem. 272: 28206-28209), myositis related inflammation, rheumatoid arthritis and joint destruction (Plater-Zyberk *et al.*, 1997 Immunol. Lett. 57: 117-120), the activation of T cells during autoimmune inflammatory diseases (Karpus *et al.*, 1997 J. Leukoc. Biol. 62: 681-687), and chronic inflammation (Wells *et al.*, 1996 J. Leukoc. Biol. 59: 53-60). Accordingly, antagonists to specific CC chemokines have been proposed as a means of regulating the conditions or disease corresponding to the CC chemokine-mediated activity.

Due to the importance of chemokines as modulators of chemotaxis and inflammation, a need exists to identify and isolate new chemokines and chemokine receptors with the potential to aid in the modulation of inflammation and the immune response. As substances that promote inflammation also may promote wound healing and angiogenesis (Gupta *et al.*, 1998 J. Biol. Chem. 273: 4282-4287), the identification

of new chemokines or chemokine-like molecules could aid in the development of angiogenic and anti-angiogenic pharmaceuticals. Modulation of inflammation is similarly important in pathological conditions manifested by inflammation. For example, Crohn's disease is manifested by chronic inflammation of all layers of the bowel and is associated with pain and diarrhea. Additionally, substances that induce an immune response may promote palliation or healing of any number of pathological conditions. Due to the important role of leukocytes (*e.g.*, neutrophils and monocytes) in cell-mediated immune responses and the established role of chemokines in leukocyte chemotaxis, the further identification, isolation and characterization of novel chemokines may thus facilitate the modulation of immune responses as well as inflammation.

SUMMARY OF THE INVENTION

The invention includes a nucleic acid molecule encoding a TSG-5 protein having the sequence of Figure 4 (SEQ ID NO. 2) or TSG-5v1 or TSG-5v2 proteins (SEQ ID NOS. 14 or 16), a nucleic acid encoding a biologically active variant of TSG-5, TSG-5v1 or TSG-5v2, a nucleic acid encoding a novel polypeptide fragment of TSG-5, TSG-5v1 or TSG-5v2, a nucleic acid molecule comprising the sequence of Figure 3 (SEQ ID NO. 1), a nucleic acid molecule, which encodes a TSG-5, TSG-5v1 or TSG-5v2 protein that hybridizes under stringent conditions to the nucleic acid, or the sense or antisense strands, depicted in SEQ ID NOS. 1, 3, 4, 13, or 15 or nucleotides 80 to 418 of SEQ ID NO. 1.

The invention also includes an isolated polypeptide comprising Figure 4 (SEQ ID NO. 2), an isolated peptide comprising the sequence of SEQ ID NOS.14 or 16, a biologically active fragment of TSG-5, TSG-5v1 or TSG-5v2, a variant of TSG-5, TSG-5v1 or TSG-5v2 or a polypeptide encoded by a nucleic acid molecule which hybridizes under stringent conditions with a probe having the nucleotide sequence of the complement of the coding sequence of Figure 3 (nucleotides 80 to 418 of SEQ ID NO. 1) or the nucleic acid sequence of SEQ ID NOs. 13 or 15.

The present invention further provides antibodies and antibody fragments that recognize and bind to epitopes of TSG-5, TSG-5v1 or TSG-5v2. The antibodies and

antibody fragments contemplated include monoclonal and polyclonal chimeric, human or humanized antibodies, as well as antibody fragments (scFv, Fab, Fab', and $F(ab')_2$).

The invention further provides methods for identifying an agent that modulates binding of TSG-5, TSG-5v1 or TSG-5v2, to a receptor or ligand, such as TNFα, comprising the steps of (1) contacting a TSG-5, TSG-5v1 or TSG-5v2 polypeptide and a TSG-5, TSG-5v1 or TSG-5v2 receptor or ligand with various concentrations of the agent; (2) assaying the specific binding of the polypeptide to the receptor or ligand; and (3) determining whether said compound modulates said specific binding.

The present invention further provides methods of imaging target sites which contain TSG-5, TSG-5v1 or TSG-5v2 in a subject comprising the steps of (1) providing a labeled TSG-5, TSG-5v1 or TSG-5v2 peptide; (2) introducing the labeled peptide into the subject; and (3) detecting the accumulated, labeled peptide thereby imaging said target site.

The present invention provides for methods of modulating chemotaxis, inflammation or cell proliferation by providing TSG-5, TSG-5v1 or TSG-5v2 agonists and antagonists.

The invention also includes methods of diagnosing an inflammatory condition or disease by ascertaining the presence of TSG-5, TSG-5v1 or TSG-5v2 in a particular organ or tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-1B. In Figure 1A, TSG-5 mRNA is detected in TNFα-treated MEFs. Northern blot analysis using the original total RNA from TNFα treated IRF-$1^{0/0}$ mouse embryonic fibroblasts (MEFs) and the TSG-5 fragments (059A14+) as a probe revealed a band of approximately 600 to 700 bp that was identified mainly in TNFα-treated IRF-1 deficient cells (Figure 1A). TSG-5 was inducible by TNFα in control wild-type cells as determined by RT-PCR (Figure 1B).

Figure 2. Restriction analysis and hybridization using the TSG-5 probe.

Figure 3. The *HindIII* fragment depicted in Figure 2 was sequenced in both orientations and revealed the presence of the originally identified TSG-5 fragment, 059A14+. The underlined sequence corresponds to the coding region of TSG-5 (nucleotides 80 to 418 of SEQ ID NO. 1).

Figure 4. Amino acid sequence alignment between TSG-5 (SEQ ID No. 2) the CC chemokine MIP3-β (SEQ ID NO. 17). The characteristic cysteines of CC chemokines are underlined, as well as the second pair of cysteines. The amino acids listed in bold represent those residues that are conserved as between TSG-5 and MIP3-β.

Figure 5. Southern blot analysis of mouse genomic DNA using a TSG-5 probe.

Figure 6. Induction of TSG-5 activity by TNFα, IFNγ or LPS in mouse macrophages. TSG-5 was demonstrated to be inducible by each of these three agents.

Figure 7. Identification of transcription factor binding sites in the TSG-5 promotor (SEQ ID No. 8). Putative transcription factor binding sites, related to TNFα and IFNα signaling, are underlined. Sites were identified by computer analysis using the program Matinspector V2.2. The "tg" repeated sequence is also found in the IL-6 promoter and in the TSG-14 promoter, two known TNFα-stimulated genes. The start codon is underlined and indicated by an arrow. Two potential TATA boxes are indicated by black boxes.

Figure 8A-8B. Intracellular staining of cells with anti-TSG-5 antibody. **Figure 8A,** Splenocytes from control or LPS-treated mice (50 μg/animal, *i.p.*, for 18 hr) were collected and single cell suspensions were prepared. For flow cytometry, cells were permeabilized and stained with a rabbit anti-TSG-5 antibody prepared against the peptide, RNHQARGEEEKGLATKDQGIC (SEQ ID No. 9), which was predicted from the ORF of the genomic sequence. A FITC-labeled goat anti-rabbit antibody was used as a secondary antibody.

Figure 8B, Thymocytes from control or LPS-treated mice (50 μg/animal, *i.p.*, for 18 hr) were collected, and single cell suspensions were prepared. For flow cytometry, cells were permeabilized and stained with a rabbit anti-TSG-5 antibody prepared against the same peptide as in Figure 10A. A FITC-labeled goat anti-rabbit antibody was again used as a secondary antibody.

Figure 9. Western Blot Detection of a TSG-5 Protein in MEFs Exposed to TNFα. Arrows indicate two molecular weight bands detected using the anti-TSG-5 antibody.

Figure 10. Rat eosinophil chemotaxis induction by TSG-5 and Eotaxin (Eot.) was measured. The Chemotaxis Index is expressed as the ratio of migrating eosinophil cells in the presence of chemokine to that of control cells. Platelet Activity Factor (PAF) was used as a positive control.

Figure 11. Sequence alignment for TSG-5v2, TSG-5v1, genomic DNA, Clone 059A14+, and TSG-5.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

1. General Description

The present invention is based in part on the identification of a gene encoding a novel murine CC chemokine, TSG-5. The present invention provides a nucleotide sequence for murine TSG-5 and the methods of using and making the murine TSG-5 DNA sequence as well as the homologous nucleic acids of related animal (*e.g.*, mammalian and human) TSG-5 DNAs.

The TSG-5 protein can serve as a target for agents that can be used to modulate the expression or activity of the protein. For example, agents may be identified which modulate biological processes associated with ribosomal activity. The present invention is further based on the development of methods for isolating binding partners that bind to

the protein, such as TSG-5 receptors. Probes based on the protein are used as capture probes to isolate potential binding partners, such as other proteins. Dominant negative proteins, DNAs encoding these proteins, antibodies to these proteins, peptide fragments of these proteins or mimics of these proteins may be introduced into cells to affect function. Additionally, these proteins provide a novel target for screening of synthetic small molecules and combinatorial or naturally occurring compound libraries to discover novel therapeutics to regulate ribosomal function.

The invention is also directed to novel nucleic acid sequences for TSG-5v1 and TSG-5v2. Polypeptides comprising amino acids encoded by the TSG-5v1 or TSG-5v2 sequences are also contemplated. Methods and uses as described for TSG-5 above are also contemplated and applicable to TSG-5v1 and TSG-5v2.

2. Definitions

"Biologically active" refers to those forms of TSG-5, polypeptides, including TSG-5, TSG-5 variants, alleles of TSG-5 or polypeptide fragments of TSG-5 which retain a measurable biological and/or immunological activity of wild-type TSG-5.

By "modulate", "regulate" and "mediate" is meant methods, conditions, or agents which increase or decrease the wild-type activity or effect of TSG-5. This change in activity may include an increase or decrease of TSG-5 mRNA translation, mRNA or DNA transcription, and/or TSG-5 protein degradation, which in turn corresponds to an increase or decrease in TSG-5 activity. Said "increase" in TSG-5 activity can be induced by means of an "agonist" which up-regulates TSG-5 activity. Likewise, "antagonists" are expected to "decrease" or "down-regulate" TSG-5 activity.

By "TSG-5 mediated activity" is meant any activity, condition, disease or phenotype which is induced or modulated by a biologically active form of a TSG-5 protein.

By "effective amount" or "dose effective amount" is meant an amount of an agent which modulates a TSG-5 mediated activity or a wild-type activity of TSG-5.

It is understood that when used in relation to TSG-5v1 or TSG-5v2, the foregoing definitions and terms shall have meanings analogous to those applied to TSG-5.

3. Nucleic Acid Molecules

The present invention further provides nucleic acid molecules that encode the TSG-5 protein of Figure 4 (SEQ ID No. 2), polypeptides comprising the amino acids of SEQ ID NOS. 14 and 16 and the related proteins herein described, preferably in isolated or purified form. As used herein, "nucleic acid" is defined as RNA, DNA, or cDNA that encodes a peptide as defined above, or is complementary to a nucleic acid sequence encoding such peptides, or hybridizes to either the sense or antisense strands of the nucleic acid and remains stably bound to it under appropriate stringency conditions. The nucleic acid may encode a polypeptide sharing at least 75% sequence identity, preferably at least 80%, and more preferably at least 85%, with the peptide sequences. Specifically contemplated are genomic DNA, cDNA, mRNA, antisense molecules, enzymatically active nucleic acids (*e.g.*, ribozymes), as well as nucleic acids based on alternative backbone or including alternative bases whether derived from natural sources or synthesized. Such hybridizing or complementary nucleic acids, however, are defined further as being novel and nonobvious over any prior art nucleic acid including that which encodes, hybridizes under appropriate stringency conditions, or is complementary to a nucleic acid encoding a protein according to the present invention.

As used herein, the terms "hybridization" (hybridizing) and "specificity" (specific for) in the context of nucleotide sequences are used interchangeably. The ability of two nucleotide sequences to hybridize to each other is based upon the degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the greater the degree of hybridization of one to the other. The degree of hybridization also depends on the conditions of stringency which include temperature, solvent ratios, salt concentrations, and the like. In particular, "selective hybridization" pertains to conditions in which the degree of hybridization of a polynucleotide of the invention to its target would require complete or nearly complete complementarity. The complementarity must be sufficiently high so as to assure that the polynucleotide of the invention will bind specifically to the target

nucleotide sequence relative to the binding of other nucleic acids present in the hybridization medium. With selective hybridization, complementarity will be 90-100%, preferably 95-100%, more preferably 100%.

"Stringent conditions" are those that (1) employ low ionic strength and high temperature for washing, for example: 0.015 M NaCl, 0.0015 M sodium titrate, 0.1% SDS at 50°C; or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is use of 50% formamide, 5X SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5X Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2X SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid from the source of nucleic acid.

The present invention further provides fragments of the encoding nucleic acid molecule. As used herein, a fragment of an encoding nucleic acid molecule refers to a small portion of the entire protein encoding sequence. The size of the fragment will be determined by the intended use. For example, if the fragment is chosen so as to encode an active portion of the protein or encode an antigenic epitope, the fragment will need to be large enough to encode the functional or antigenic region(s) of the protein. If the fragment is to be used as a nucleic acid probe or PCR primer, then the fragment length is chosen so as to obtain a relatively small number of false positives during probing/priming.

Fragments of the encoding nucleic acid molecules of the present invention (*i.e.*, synthetic oligonucleotides) that are used as probes or specific primers for the polymerase chain reaction (PCR), or to synthesize gene sequences encoding proteins of the invention can easily be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci *et al.*, (J. Am. Chem. Soc. 103: 3185-3191, 1981) or using automated synthesis

methods. In addition, larger DNA segments can readily be prepared by well known methods, such as synthesis of a group of oligonucleotides that define various modular segments of the gene, followed by ligation of oligonucleotides to build the complete modified gene.

The TSG-5 encoding nucleic acid molecules of the present invention may further be modified to contain a detectable label for diagnostic and probe purposes. A variety of such labels are known in the art and can readily be employed with the encoding molecules herein described. Suitable labels include, but are not limited to, biotin, radiolabeled nucleotides and the like. A skilled artisan can employ any of the art known labels to obtain a labeled encoding nucleic acid molecule.

Modifications to the primary structure itself by deletion, addition, or alteration of the amino acids incorporated into the protein sequence during translation can be made without destroying the activity of the protein. Such substitutions or other alterations result in proteins having an amino acid sequence encoded by a nucleic acid falling within the contemplated scope of the present invention.

Antisense molecules corresponding to the TSG-5 coding or non-coding sequence may be prepared. Methods of making antisense molecules which bind to TSG, form triple helices or are enzymatically active and cleave TSG RNA and single stranded DNA (ssDNA) are known in the art. See ANTISENSE AND RIBOZYME METHODOLOGY: LABORATORY COMPANION (Ian Gibson, ed., Chapman & Hall 1997) and RIBOZYME PROTOCOLS: METHODS IN MOLECULAR BIOLOGY (Phillip C. Turner, ed., Humana Press, Clifton, NJ 1997).

4. **Isolation of Other Related Nucleic Acid Molecules**

As described above, the identification of the murine nucleic acid molecules of SEQ ID NOS. 1, 13 or 15 allows a skilled artisan to isolate nucleic acid molecules that encode other members of the TSG-5 CC chemokine family. This would include the isolation of other mammalian TSG-5 sequences, including human TSG-5. Further, the presently disclosed nucleic acid molecules allow a skilled artisan to isolate nucleic acid molecules that encode TSG-5-like proteins, in addition to the disclosed protein of Figure 4 (SEQ ID NO. 2) or TSG-5v1 or TSG-5v2 polypeptides described herein (SEQ ID NOS. 14 or 16).

A skilled artisan can readily use the amino acid sequence of Figure 4 (SEQ ID NO. 2) , or SEQ ID NOS. 14 or 16, to generate antibody probes to screen expression libraries prepared from appropriate cells. Typically, polyclonal antiserum from mammals such as rabbits immunized with the purified protein (as described below) or monoclonal antibodies can be used to probe a mammalian cDNA or genomic expression library, such as a human macrophage library, to obtain the appropriate coding sequence for other members of the protein family. The cloned cDNA sequence can be expressed as a fusion protein, expressed directly using its own control sequences, or expressed by constructions using control sequences appropriate to the particular host used for expression of the TSG-5 protein.

Alternatively, a portion of the coding sequence herein described can be synthesized and used as a probe to retrieve DNA encoding a member of the protein family from any mammalian organism. Oligomers containing approximately 18-20 nucleotides (encoding about a 6-7 amino acid
stretch) are prepared and used to screen genomic DNA or cDNA libraries to obtain hybridization under stringent conditions or conditions of sufficient stringency to eliminate an undue level of false positives.

Additionally, pairs of oligonucleotide primers can be prepared for use in a polymerase chain reaction (PCR) to selectively clone an encoding nucleic acid molecule. A PCR denature/anneal/extend cycle for using such PCR primers is well known in the art and can readily be adapted for use in isolating other encoding nucleic acid molecules. For example, degenerate primers can be utilized to obtain sequences related to TSG-5. Primers can be designed that are not perfectly complementary and can still hybridize to a portion of a target sequence or flanking sequence and thereby provide for amplification of all or a portion of a target sequence. Primers of about 20 nucleotides or less, preferably have about one to three mismatches located at the 5' and/or 3' ends. Primers of about 20 to 30 nucleotides have up to about 30% mismatches and can still hybridize to a target sequence. Hybridization conditions for primers with mismatch can be determined by the method described in Maniatis *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982) or by reference to known methods. The ability of the primer to hybridize to a sequence of SEQ ID NOS. 1, 3, 4, 7, 13 or 15, or a related sequence, under

varying conditions can be determined using this method. Because a target sequence is known, the effect of mismatches can be determined by methods known to those of skill in the art. Degenerate primers would be based on putative conserved amino acid sequences of the TSG-5 gene.

5. rDNA Molecules Encoding a TSG-5 Nucleic Acid

The present invention further provides recombinant DNA molecules (rDNAs) that contain a TSG-5 coding sequence. As used herein, a rDNA molecule is a DNA molecule that has been subjected to molecular manipulation *in situ*. Methods for generating rDNA molecules are well known in the art, for example, see Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). In the preferred rDNA molecules, a coding DNA sequence is operably linked to expression control sequences and/or vector sequences.

The choice of vector and/or expression control sequences to which one of the protein family encoding sequences of the present invention is operably linked depends directly, as is well known in the art, on the functional properties desired, *e.g.*, protein expression, and the host cell to be transformed. A vector contemplated by the present invention is at least capable of directing the replication or insertion into the host chromosome, and preferably also expression, of the structural gene included in the rDNA molecule.

Expression control elements that are used for regulating the expression of an operably linked protein encoding sequence are known in the art and include, but are not limited to, inducible promoters, constitutive promoters, secretion signals, and other regulatory elements. Preferably, the inducible promoter is readily controlled, such as being responsive to a nutrient in the host cell's medium.

In one embodiment, the vector containing a coding nucleic acid molecule will include a prokaryotic replicon, *i.e.*, a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, vectors with a prokaryotic replicon may also include a

gene whose expression confers a detectable marker such as a drug resistance. Typical bacterial drug resistance genes are those that confer resistance to ampicillin or tetracycline.

Vectors that include a prokaryotic replicon can further include a prokaryotic or bacteriophage promoter capable of directing the expression (transcription and translation) of the coding gene sequences in a bacterial host cell, such as *E. coli*. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA), pPL and pKK223 available from Pharmacia (Piscataway, NJ).

Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can also be used to form a rDNA molecule that contains a coding sequence. Eukaryotic cell expression vectors are well known in the art and are available from several commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of a desired DNA segment. Typical of such vectors are pSVL and pKSV-10 (Pharmacia), pBPV-1/pML2d (International Biotechnologies, Inc.), vector systems that include Histidine Tags and periplasmic secretion, or other vectors described in the art.

Eukaryotic cell expression vectors used to construct the rDNA molecules of the present invention may further include a selectable marker that is effective in an eukaryotic cell, preferably a drug resistance selection marker. A preferred drug resistance marker is the gene whose expression results in neomycin resistance, *i.e.*, the neomycin phosphotransferase (*neo*) gene (Southern *et al.*, 1982 J. Mol. Anal. Genet. 1: 327-341). Alternatively, the selectable marker can be present on a separate plasmid, and the two vectors introduced by co-transfection of the host cell, and selected by culturing in the appropriate drug for the selectable marker.

6. Host Cells Containing an Exogenously Supplied TSG-5, TSG-5v1 or TSG-5v2 Coding Nucleic Acid Molecule

The present invention further provides host cells transformed with a nucleic acid molecule that encodes a TSG-5, TSG-5v1 or TSG-5v2 protein of the present invention. The host cell can be either prokaryotic or eukaryotic. Eukaryotic cells useful for expression of a protein of the invention are not limited, so long as the cell line is compatible with cell culture methods and compatible with the propagation of the expression vector and expression of the gene product. Preferred eukaryotic host cells include, but are not limited to, yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, baby hamster kidney cells (BHK), and the like eukaryotic tissue culture cell lines.

Any prokaryotic host can be used to express a rDNA molecule encoding a protein of the invention. The preferred prokaryotic host is *E. coli*.

Transformation of appropriate cell hosts with a rDNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used and host system employed. With regard to transformation of prokaryotic host cells, electroporation and salt treatment methods are typically employed; see, for example, Cohen *et al.*, Proc. Natl. Acad. Sci. USA 69: 2110, 1972; and Maniatis *et al.*, (1982). With regard to transformation of vertebrate cells with vectors containing rDNAs, electroporation, cationic lipid or salt treatment methods are typically employed; see, for example, Graham *et al.*, Virol. 52: 456, 1973; Wigler *et al.*, Proc. Natl. Acad. Sci. USA 76: 1373-76, 1979.

Successfully transformed cells, *i.e.*, cells that contain a rDNA molecule of the present invention, can be identified by well known techniques including the selection for a selectable marker. For example, cells resulting from the introduction of an rDNA of the present invention can be cloned to produce single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, 1975 J. Mol. Biol. 98: 503, or Berent *et al.*, 1985 Biotech. 3: 208, or the proteins produced from the cell assayed via an immunological method.

Recombinant TSG-5, TSG-5v1 or TSG-5v2 DNA can also be utilized to analyze the function of coding and non-coding sequences. Sequences that modulate the translation of the TSG-5, TSG-5v1 or TSG-5v2 mRNA can be utilized in an affinity matrix system to purify proteins obtained from cell lysates that associate with the TSG-5, TSG-5v1 or TSG sequence. Synthetic oligonucleotides would be coupled to the beads and probed with the lysates, as is commonly known in the art. Associated proteins could then be separated using, for example, a two dimensional SDS-PAGE system. Proteins thus isolated could be further identified using mass spectroscopy or protein sequencing.

7. Production of Recombinant TSG-5, TSG-5v1 or TSG-5v2 Peptides Proteins using a cDNA or Other Recombinant Nucleic Acids

The invention also relates to nucleic acid molecules which encode a TSG-5, TSG-5v1 or TSG-5v2 protein and polypeptide fragments thereof and analog molecules. The TSG-5, TSG-5v1 or TSG-5v2 polypeptides include a full length TSG-5, TSG-5v1 or TSG-5v2 protein, preferably that is mammalian and most preferably that is human. Nucleic acid molecules encoding polypeptide fragments having a consecutive amino acid sequence of at least 3, 5, 10, 15, 20, 25, 30 or 40 amino acid residues from a common TSG-5, TSG-5v1 or TSG-5v2 sequence; amino acid sequence variants of a common TSG-5, TSG-5v1 or TSG-5v2 sequence wherein an amino acid residue has been inserted N- or C-terminal to, or within, the TSG-5, TSG-5v1 or TSG-5v2 sequence or its fragments; amino acid sequence variants of the common TSG-5, TSG-5v1 or TSG-5v2 sequence or its fragment, which have been substituted by another conserved residue. TSG-5, TSG-5v1 or TSG-5v2 nucleic acid molecules which encode polypeptides include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and TSG-5, TSG-5v1 or TSG-5v2 polypeptides of other animal species, including but not limited to vertebrates (*e.g.*, rabbit, rat, murine, porcine, bovine, ovine, equine and non-human primate species) as well as invertebrates, and alleles or other naturally occurring variants of TSG-5, TSG-5v1 or TSG-5v2 of the foregoing species and of human sequences; derivatives of the commonly known TSG-5, TSG-5v1 or TSG-5v2 or its fragments, wherein TSG-5, TSG-5v1 or TSG-5v2 or its fragments have been covalently modified by substitution, chemical, enzymatic, or other

appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope); and soluble forms of TSG-5, TSG-5v1 or TSG-5v2.

The novel nucleic acid molecules encoding a TSG-5,TSG-5v1 or TSG-5v2 protein and polypeptides of the present invention are preferably those which share a common biological activity (*e.g.*, CC chemokine-like activity) with TSG-5, TSG-5v1 or TSG-5v2. The present TSG-5, TSG-5v1 or TSG-5v2 proteins, analogs, fragments and variants exclude other known CC chemokines which are not TSG-5, TSG-5v1 or TSG-5v2-like. The TSG-5, TSG-5v1 or TSG-5v2 polypeptides include those encoded by a nucleic acid molecule with silent mutations, as well as those nucleic acids encoding a TSG-5, TSG-5v1 or TSG-5v2 protein with conservative amino acid substitutions, allelic variants, and other variants of TSG-5, TSG-5v1 or TSG-5v2 with CC chemokine-like activity.

The compounds of the invention are TSG-5, TSG-5v1 or TSG-5v2 peptides which are partially defined in terms of amino acid residues of designated classes. TSG-5, TSG-5v1 or TSG-5v2 homologues would include conservative amino acid substitutions within the amino acid classes described below. Amino acid residues can be generally subclassified into four major subclasses as follows:

Acidic: The residue has a negative charge due to loss of H^+ ion at physiological pH, and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium, at physiological pH.

Basic: The residue has a positive charge due to association with H^+ ion at physiological pH, and the residue is attracted by aqueous solution so as to seek the surface positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium at physiological pH.

Neutral/non-polar: The residues are not charged at physiological pH, but the residue is repulsed by aqueous solution so as to seek the inner positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium. These residues are also designated "hydrophobic."

Neutral/polar: The residues are not charged at physiological pH, but the residue is attracted by aqueous solution so as to seek the outer positions in the conformation of a peptide in which it is contained when the peptide is in aqueous medium.

It is understood, of course, that in a statistical collection of individual residue molecules some molecules will be charged, and some not, and there will be an attraction for or repulsion from an aqueous medium to a greater or lesser extent. To fit the definition of "charged", a significant percentage (at least approximately 25%) of the individual molecules are charged at physiological pH. The degree of attraction or repulsion required for classification as polar or nonpolar is arbitrary and, therefore, amino acids specifically contemplated by the invention have been classified as one or the other. Most amino acids not specifically named can be classified on the basis of known behavior.

Amino acid residues can be further subclassified as cyclic or noncyclic, and aromatic or non-aromatic, self-explanatory classifications with respect to the side chain substituent groups of the residues, and as small or large. The residue is considered small if it contains a total of 4 carbon atoms or less, inclusive of the carboxyl carbon. Small residues are, of course, always nonaromatic.

The gene-encoded secondary amino acid proline, although technically within the group neutral/nonpolar/large/cyclic and nonaromatic, is a special case due to its known effects on the secondary conformation of peptide chains, and is not, therefore, included in this defined group.

Other amino acid substitutions of those encoded in the gene can also be included in peptide compounds within the scope of the invention and can be classified within this general scheme according to their structure.

All of the compounds of the invention, when an amino acid forms the C-terminus, may be in the form of the pharmaceutically acceptable salts or esters. Salts may be, for example, Na^+, K^+, Ca^{+2}, Mg^{+2} and the like, the esters are generally those of alcohols of 1-6 carbons.

The present invention further provides methods for producing a protein of the invention using nucleic acid molecules herein described. In general terms, the production of a recombinant form of a protein typically involves the following steps:

First, a nucleic acid molecule is obtained that encodes a protein of the invention, such as the nucleic acid molecule depicted in SEQ ID NOS. 1 or particularly for TSG-5, the nucleotides 80 to 418 of SEQ ID NO. 1, which encode the TSG-5 open reading frame (ORF). This coding sequence (ORF) is directly suitable for expression in any host, as it is not interrupted by introns.

These DNAs can be transfected into host cells such as eukaryotic cells or prokaryotic cells. Eukaryotic hosts include mammalian cells (*e.g.*, mouse embryonic fibroblasts (MEFs), macrophages and T cells or in transgenic animals, as well as insect cells, such as Sf9 cells using recombinant baculovirus. For example, a DNA expressing an ORF under control of a T cell specific promoter *(e.g.*, the LCK promoter) can be inserted into a mouse to create a transgenic animal model. Alternatively, TSG-5 can be expressed in an heterologous system. The human cell line GM637, SV-40 transformed human fibroblasts, can be transfected, with a plasmid containing a TSG-5 coding sequence under the control of the chicken actin promoter (Reis *et al.*, 1992 EMBO J. 11: 185-193). Such transfected cells could be used as a source of TSG-5 in functional assays. Alternatively, fragments encoding only a portion of TSG-5 can be expressed alone or in the form of a fusion protein. For example, the TSG-5 ORF can be expressed in bacteria (*e.g.*, *E. coli*) as a GST- or His-Tag fusion protein. These fusion proteins are then purified and can be used to generate polyclonal antibodies.

The nucleic acid molecule is then preferably placed in operable linkage with suitable control sequences, as described above, to form an expression unit containing the protein open reading frame. The expression unit is used to transform a suitable host and the transformed host is cultured under conditions that allow the production of the recombinant protein. Optionally the recombinant protein is isolated from the medium or from the cells; recovery and purification of the protein may not be necessary in some instances where some impurities may be tolerated.

Each of the foregoing steps can be done in a variety of ways and can be applied to TSG-5, TSG-5v1 or TSG-5v2 as described for TSG-5 above. For example, the desired coding sequences may be obtained from genomic fragments and used directly in appropriate hosts. The construction of expression vectors that are operable in a variety of hosts is accomplished using appropriate replicons and control sequences, as set forth above. The

control sequences, expression vectors, and transformation methods are dependent on the type of host cell used to express the gene and were discussed in detail earlier. Suitable restriction sites can, if not normally available, be added to the ends of the coding sequence so as to provide an excisable gene to insert into these vectors. A skilled artisan can readily adapt any host/expression system known in the art for use with the nucleic acid molecules of the invention to produce recombinant protein.

8. **Methods to Identify Binding Partners**

Another embodiment of the present invention provides methods for use in isolating and identifying binding partners of proteins (*e.g.*, TSG-5, TSG-5v1 or TSG-5v2 receptors) of the invention In detail, a protein of the invention is mixed with a potential binding partner or an extract or fraction of a cell under conditions that allow the association of potential binding partners with the protein of the invention. After mixing, peptides, polypeptides, proteins or other molecules that have become associated with a protein of the invention are separated from the mixture. The binding partner that bound to TSG-5, for example, or a fragment thereof can then be removed and further analyzed. To identify and isolate a binding partner, the entire protein, for instance the entire disclosed protein of SEQ ID NO. 2, can be used. Partial amino acid sequences, such as SEQ ID NOS. 14 and 16 can also be used. Alternatively, a fragment of the protein can be used.

As used herein, a cellular extract refers to a preparation or fraction which is made from a lysed or disrupted cell.

A variety of methods can be used to obtain cell extracts. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with the protein of the invention under conditions in which association of the protein with the binding partner can occur. A variety of conditions can be used, the most preferred being conditions that closely

resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to a protein of the invention can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

To aid in separating associated binding partner pairs from the mixed extract, the protein of the invention can be immobilized on a solid support. For example, the protein can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the protein to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins.

Alternatively, the nucleic acid molecules of the invention can be used in a yeast two-hybrid system. The yeast two-hybrid system has been used to identify other protein partner pairs and can readily be adapted to employ the nucleic acid molecules herein described.

One preferred *in vitro* binding assay for TSG-5 would comprise a mixture of a TSG-5 polypeptide and one or more candidate binding targets or substrates. After incubating the mixture under appropriate conditions, one would determine whether TSG-5 or a polypeptide fragment thereof bound with the candidate substrate. For cell-free binding assays, one of the components usually comprises or is coupled to a label. The label may provide for direct detection, such as radioactivity, luminescence, optical or electron density, *etc.*, or indirect detection such as an epitope tag, an enzyme, *etc.* A variety of methods may be employed to detect the label depending on the nature of the label and other assay components. For example, the label may be detected bound to the solid substrate or a portion of the bound

complex containing the label may be separated from the solid substrate, and the label thereafter detected.

9. **Methods to Identify Agents that Modulate the Expression of a Nucleic Acid Encoding the TSG-5, TSG-5v1 or TSG-5v2 Protein**

Another embodiment of the present invention provides methods for identifying agents that modulate the expression of a nucleic acid encoding a protein of the invention such as a protein having the amino acid sequence of SEQ ID NOS. 2, 14 or 16. Such assays may utilize any available means of monitoring for changes in the expression level of the nucleic acids of the invention. As used herein, an agent is said to modulate the expression of a nucleic acid of the invention, for instance a nucleic acid encoding the protein having the sequence of SEQ ID NOS. 2, 14 or 16, if it is capable of up- or down-regulating expression of the nucleic acid in a cell (*e.g.*, mRNA).

In one assay format, cell lines that contain reporter gene fusions between the open reading frame defined, for example, by TSG-5, TSG-5v1 or TSG-5v2 nucleic acids. For example, nucleotides 1419-1757 of SEQ ID NO. 3, and any assayable fusion partner may be prepared. Numerous assayable fusion partners are known and readily available including the firefly luciferase gene and the gene encoding chloramphenicol acetyltransferase (Alam *et al.*, 1990 Anal. Biochem. 188: 245-254). Cell lines containing the reporter gene fusions are then exposed to the agent to be tested under appropriate conditions and time. Differential expression of the reporter gene between samples exposed to the agent and control samples identifies agents which modulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID NO. 2, or depending on the nucleic and selected SEQ ID Nos. 14 or 16.

Additional assay formats may be used to monitor the ability of the agent to modulate the expression of a nucleic acid encoding a protein of the invention such as the protein of SEQ ID NOS. 2, 14 or 16. For instance, mRNA expression may be monitored directly by hybridization to the nucleic acids of the invention. Cell lines are exposed to the agent to be tested under appropriate conditions and time and total RNA or mRNA is isolated by standard procedures such those disclosed in Sambrook *et al.* (1989).

Probes to detect differences in RNA expression levels between cells exposed to the agent and control cells may be prepared from the nucleic acids of the invention. It is preferable, but not necessary, to design probes which hybridize only with target nucleic acids under conditions of high stringency. Only highly complementary nucleic acid hybrids form under conditions of high stringency. Accordingly, the stringency of the assay conditions determines the amount of complementarity which should exist between two nucleic acid strands in order to form a hybrid. Stringency should be chosen to maximize the difference in stability between the probe:target hybrid and potential probe:non-target hybrids.

Probes may be designed from the nucleic acids of the invention through methods known in the art. For instance, the G+C content of the probe and the probe length can affect probe binding to its target sequence. Methods to optimize probe specificity are commonly available in Sambrook *et al.* (1989) or Ausubel *et al.* (CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene Publishing Co., NY, 1995).

Hybridization conditions are modified using known methods, such as those described by Sambrook *et al.* (1989) and Ausubel *et al.* (1995) as required for each probe. Hybridization of total cellular RNA or RNA enriched for polyA RNA can be accomplished in any available format. For instance, total cellular RNA or RNA enriched for polyA RNA can be affixed to a solid support and the solid support exposed to at least one probe comprising at least one, or part of one of the sequences of the invention under conditions in which the probe will specifically hybridize. Alternatively, nucleic acid fragments comprising at least one, or part of one of the sequences of the invention can be affixed to a solid support, such as a porous glass wafer. The glass or silica wafer can then be exposed to total cellular RNA or polyA RNA from a sample under conditions in which the affixed sequences will specifically hybridize. Such glass wafers and hybridization methods are widely available, for example, those disclosed by Beattie (WO 95/11755). By examining for the ability of a given probe to specifically hybridize to an RNA sample from an untreated cell population and from a cell population exposed to the agent, agents which up or down regulate the expression of a nucleic acid encoding the protein having the sequence of SEQ ID NOS. 2, 14 or 16 are identified.

10. **Methods to Identify Agents that Modulate at Least One Activity of TSG-5, TSG-5v1 or TSG-5v2 and Related Antibodies**

Another embodiment of the present invention provides methods for identifying agents that modulate at least one activity of a TSG-5, TSG-5v1 or TSG-5v2 or related protein, such as the protein having the amino acid sequence of SEQ ID NO. 2, 14 or 16. Such methods or assays may utilize any means of monitoring or detecting the desired activity.

In one format, the relative amounts of a protein of the invention between a cell population that has been exposed to the agent to be tested compared to an un-exposed control cell population may be assayed. In this format, probes such as specific antibodies are used to monitor the differential expression of the protein in the different cell populations. Cell lines or populations are exposed to the agent to be tested under appropriate conditions and time. Cellular lysates may be prepared from the exposed cell line or population and a control, unexposed cell line or population. The cellular lysates are then analyzed with the probe.

For example, N- and C- terminal fragments of TSG-5, TSG-5v1 or TSG-5v2 can be expressed in bacteria and used to search for proteins which bind to these fragments. Fusion proteins, such as His-tag or GST fusion to the N- or C-terminal regions of TSG-5, TSG-5v1 or TSG-5v2 or a whole TSG-5, TSG-5v1 or TSG-5v2 protein can be prepared. These fusion proteins can be coupled to Talon or Glutathione-Sepharose beads and then probed with cell lysates. Prior to lysis, the cells may be treated with rapamycin or other drugs which may modulate TSG-5, TSG-5v1 or TSG-5v2 or proteins that interact with TSG-5, TSG-5v1 or TSG-5v2. Lysate proteins binding to the fusion proteins can be resolved by SDS-PAGE, isolated and identified by protein sequencing or mass spectroscopy, as is known in the art.

Antibody probes are prepared by immunizing suitable mammalian hosts in appropriate immunization protocols using the peptides, polypeptides or proteins of the invention if they are of sufficient length, or, if desired, or if required to enhance immunogenicity, they can be conjugated to suitable carriers. Methods for preparing immunogenic conjugates with carriers such as bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be effective; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford,

IL, may be desirable to provide accessibility to the hapten. The hapten peptides can be extended at either the amino or carboxy terminus with a cysteine residue or interspersed with cysteine residues, for example, to facilitate linking to a carrier. Administration of the immunogens is conducted generally by injection over a suitable time period and with use of suitable adjuvants, as is generally understood in the art. During the immunization schedule, titers of antibodies are taken to determine adequacy of antibody formation.

Anti-peptide antibodies can be generated using synthetic peptides, for example, from the TSG-5 peptide RNHQARGEEEKGLATKDQGIC. (SEQ ID No. 9). Synthetic peptides can be as small as 2-3 amino acids in length, but are preferably at least 3, 5, 10, or 15 or more amino acid residues long. Such peptides can be determined using programs such as DNAStar. The peptides are coupled to KLH using standard methods and can be immunized into animals such as rabbits. Polyclonal anti-TSG-5, TSG-5v1 or TSG-5v2 peptide antibodies can then be purified, for example using Actigel beads containing the covalently bound peptide.

While the polyclonal antisera produced in this way may be satisfactory for some applications, for pharmaceutical compositions, use of monoclonal preparations is preferred. Immortalized cell lines which secrete the desired monoclonal antibodies may be prepared using the standard method of Kohler and Milstein or modifications which effect immortalization of lymphocytes or spleen cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the peptide hapten, polypeptide or protein. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells can be cultured either *in vitro* or by production in ascites fluid.

The desired monoclonal antibodies are then recovered from the culture supernatant or from the ascites supernatant. Fragments of the monoclonals or the polyclonal antisera which contain the immunologically significant portion can be used as agonists or antagonists of TSG-5, TSG-5v1 or TSG-5v2 activity, as well as the intact antibodies. Use of immunologically reactive fragments, such as the Fab, scFV Fab', of $F(ab')_2$ fragments are often preferable, especially in a therapeutic context, as these fragments are generally less immunogenic than the whole immunoglobulin.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of receptor can also be produced in the context of chimeras with multiple species origin. Antibody reagents so created are contemplated for use diagnostically or as stimulants or inhibitors of TSG-5, TSG-5v1 or TSG-5v2.

"Chimeric" antibodies are encoded by immunogoblin genes that have been genetically engineered so that the light and heavy chain genes are composed of immunogoblin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments. Such a chimeric antibody is likely to be less antigenic to a human than antibodies with mouse constant regions as well as mouse variable regions.

As used herein, the term chimeric antibody also refers to an antibody that includes an immunogoblin that has a human-like framework and in which any constant region present has at least about 85-90%, and preferably about 95% polypeptide sequence identity to a human immunogoblin (see, for example, PCT Publication WO 90/07861, which is incorporated herein by reference). Hence, all parts of such a "humanized" immunogoblin, except possibly the complementarily determining regions (CDR's), are substantially identical to corresponding parts of none or more native human immunogoblin sequences. Where necessary, framework residues may also be replaced with those within or across species especially if certain framework residues are found to affect the structure of the CDRs. A chimeric antibody may also contain truncated variable or constant regions.

Agents that are assayed in the above method can be randomly selected or rationally selected or designed. As used herein, an agent is said to be randomly selected when the agent is chosen randomly without considering the specific sequences involved in the association of the a protein of the invention alone or with its associated substrates, binding partners, etc. An example of randomly selected agents is the use a chemical library or a peptide combinatorial library, or a growth broth of an organism.

The agents of the present invention can be, as examples, peptides, small molecules, vitamin derivatives, as well as carbohydrates. A skilled artisan can readily recognize that there is no limit as to the structural nature of the agents of the present invention.

The peptide agents of the invention can be prepared using standard solid phase (or solution phase) peptide synthesis methods, as is known in the art. In addition, the DNA encoding these peptides may be synthesized using commercially available oligonucleotide synthesis instrumentation and produced recombinantly using standard recombinant production systems. The production using solid phase peptide synthesis is necessitated if non-nucleic acid-encoded amino acids are to be included. Another class of agents of the present invention are antibodies immunoreactive with critical positions of proteins of the invention. Antibody agents are obtained by immunization of suitable mammalian subjects with peptides, containing as antigenic regions, those portions of the protein intended to be targeted by the antibodies.

11. Uses for Agents that Modulate Expression or at Least One Activity of TSG-5, TSG-5v1 or TSG-5v2

As provided in the Examples, the proteins and nucleic acids of the invention, such as the protein having the amino acid sequence of SEQ ID NOs. 2, 14 or 16, are involved in inflammatory and immune responses. Agents that modulate or down-regulate the expression of the protein or agents, such as agonists or antagonists of at least one activity, of the protein may be used to modulate biological and pathologic processes associated with the protein's function and activity. The use of antibodies to modulate protein activity is specifically contemplated. Humanized antibodies, because of the lower incidence of immune reaction, are particular useful for therapeutic uses.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of a pathological or biological process mediated by a protein of the invention. The term "mammal" is meant an individual belonging to the class *Mammalia*. The invention is particularly useful in the treatment of human subjects.

As used herein, a biological or pathological process mediated by a protein of the invention may include binding of TSG-5, TSG-5v1 or TSG-5v2 to a TSG-5, TSG-5v1 or TSG-5v2 receptor or ligand, inhibiting TSG-5 mRNA synthesis or inhibiting inducers of TSG-5, TSG-5v1 or TSG-5v2 synthesis.

Pathological processes refer to a category of biological processes which produce a deleterious effect. For example, expression or up-regulation of expression of a protein of the invention may be associated with certain diseases or pathological conditions. As used herein, an agent is said to modulate a pathological process when the agent reduces the degree or severity of the process. For instance, a disease or pathological condition may be prevented, or disease progression modulated by the administration of agents which reduce or modulate in some way the expression or at least one activity of a protein of the invention.

As CC chemokines are involved both directly and indirectly in inflammation, one embodiment of this invention is to use peptides and nucleic acids of the invention, for example, TSG-5, as a method of diagnosing an inflammatory condition or disease. Certain CC chemokines are associated with specific inflammatory conditions (*e.g.*, eotaxin is a specific chemoattractant for eosinophils and is implicated in the pathogenesis of eosinophilic inflammatory diseases such as asthma; see Garcia-Zepeda *et al.*, 1997 Genomics 41: 471-476). Diagnostic tests for inflammatory conditions may include the steps of testing a sample or an extract thereof for the presence of TSG-5, TSG-5v1 or TSG-5v2 DNA, oligomers or fragments thereof or TSG-5, TSG-5v1 or TSG-5v2 protein. For example, standard *in situ* hybridization or other imaging techniques can be utilized to diagnose an inflammatory condition.

This invention also relates to methods of modulating inflammation or inflammatory conditions. Inhibition of inflammation can occur by inhibiting the chemotactic ability of the chemokine or its direct inflammatory activities. For example, one CC chemokine, eotaxin, is not only a chemoattractant, but it also has the capacity to induce the release of reactive oxygen species which directly damages tissue and leads to further inflammation (Elsner *et al.*, 1996 Eur. J. Immunol. 26: 1919-1925). Therefore, modulation of eotaxin's ability to release reactive oxygen species could inhibit its direct inflammatory activity. Modulation of TSG-5, TSG-5v1 or TSG-5v2 via agonists and antagonists could likewise regulate its ability to directly or indirectly (as a chemoattractant) cause inflammation or tissue damage. Such modulation of TSG-5, TSG-5v1 or TSG-5v2 includes inhibition of the activity of the TSG-5, TSG-5v1 or TSG-5v2 protein, transcription of the *TSG-5, TSG-5v1* or *TSG-5v2* gene or translation of TSG-5, TSG-5v1 or TSG-5v2 mRNA.

Another pathological process that may be modulated by changing the levels or activity of TSG-5, TSG-5v1 or TSG-5v2 is chemotaxis. CC chemokines are known for their ability to attract immune cells. Instances will exist when the attraction of immune cells to a site of infection would be beneficial to the subject (*e.g.*, when the subject is immunosuppressed). In other diseases (*e.g.*, asthma and rheumatoid arthritis), it would be beneficial to reduce inflammation, which would be aided by the inhibition of chemotaxis by CC chemokines. One class of agents that modulate TSG-5, TSG-5v1 or TSG-5v2 are those which modulate the chemotactic ability of TSG-5, TSG-5v1 or TSG-5v2. Agonists or antagonists of TSG-5, TSG-5v1 or TSG-5v2 therefore could be used to modulate TSG-5, TSG-5v1 or TSG-5v2 induced chemotaxis. Chemotaxis can be assayed in cells exposed to recombinant TSG-5, TSG-5v1 or TSG-5v2 protein or in a transgenic animal model with an inducible TSG-5, TSG-5v1 or TSG-5v2 gene.

This invention also relates to a method of modulating the proliferation of cells by regulating TSG-5, TSG-5v1 or TSG-5v2. Certain CC chemokines have been identified which, for example, upregulate T- cell activity and proliferation (Karpus *et al.*, 1997 J. Leukoc. Biol. 62: 681-687). Differentiation of cells, such as T cells, can be ascertained by changes in the markers expressed by the T cells (*e.g.*, the change in markers expressed on Th0 cells versus those expressed on Th2 cells). Modulation of TSG-5, TSG-5v1 or TSG-5v2 may aid in the regulation of activation, proliferation, and/or differentiation of certain immune cell populations. Agonists and antagonists of TSG-5 could be administered to a subject to modulate cellular proliferation, activation of cells and the differentiation of cells such as cellular proliferation or differentiation of cells during the administration of chemotherapy to a patient.

The agents of the present invention can be provided alone, or in combination with other agents that modulate a particular pathological process. For example, an agent of the present invention can be administered in combination with anti-thrombotic agents. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time.

The agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, transdermal or buccal routes. Alternatively, or concurrently, administration may be by the oral route. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The present invention further provides compositions containing one or more agents which modulate expression or at least one activity of a protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise from about 0.1 to about 500 μg/kg body weight. The preferred dosages comprise from about 0.1 to about 10 μg/kg body weight. The most preferred dosages comprise from about 0.1 to about 1 μg/kg body weight. In an average human of 70 kg, the range would be from about 7 μg to about 35 mg, with a preferred range of about 0.5 μg to about 5 mg.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, (*e.g.*, ethyl oleate or triglycerides). Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes and other non-viral vectors can also be used to encapsulate the agent for delivery into the cell.

The pharmaceutical formulation for systemic administration according to the invention may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulations may be used simultaneously to achieve systemic administration of the active ingredient.

Suitable formulations for oral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In practicing the methods of this invention, the compounds of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be coadministered along with other compounds typically prescribed for these conditions according to generally accepted medical practice. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

12. Transgenic Animals

Transgenic animal models can be created which conditionally express TSG-5 to study the physiological effects of TSG-5, TSG-5v1 or TSG-5v2 modulate TSG-5, TSG-5v1 or TSG-5v2 activity *in vivo*, and determine efficacy of candidate compounds which modulate TSG-5, TSG-5v1 or TSG-5v2 activity *in vitro*. Animals, such as transgenic mice, can be created using the techniques employed to make transgenic mice that express the IP-10 chemokine (Luster *et al.*, 1998 Proc. Assoc. Am. Physicians 110: 183-196), MCP-1 (Grewal *et al.*, 1997 J. Immunol. 159: 401-408) or to study chemokine function in the lung (Lira *et al.*, 1997 Methods Enzymol. 287: 304-318). General methods for creating transgenic animals are known in the art, and are described in, for example, STRATEGIES IN TRANSGENIC ANIMAL SCIENCE (Glenn M. Monastersky and James M. Robl eds., ASM Press; Washington, DC, 1995); TRANSGENIC ANIMAL TECHNOLOGY: A LABORATORY HANDBOOK (Carl A. Pinkert ed., Academic Press 1994); TRANSGENIC ANIMALS (Louis Marie Houdebine, ed., Harwood Academic Press, 1997); OVEREXPRESSION AND KNOCKOUT OF CYTOKINES IN TRANSGENIC MICE (Chaim O. Jacob, ed., Academic Press 1994); MICROINJECTION AND TRANSGENESIS: STRATEGIES AND PROTOCOLS (Springer Lab Manual) (Angel Cid-Arregui and Alejandro Garcia-Carranca, eds., Springer Verlag 1998); and MANIPULATING THE MOUSE EMBRYO: A LABORATORY MANUAL (Brigid Hogan *et al.*, editors, Cold Spring Harbor Laboratory Press 1994).

13. ***In Vivo* Imaging Using TSG-5, TSG-5v1 or TSG-5v2**

Another embodiment of this invention is the use of the TSG-5,TSG-5v1 or TSG-5v2 or a polypeptide fragment thereof that is tagged with an imaging agent as a means of determining localized expression of the said protein and its activity. The methods of performing such imaging may be similar to those described for the labeling of chemokines discussed in Lyle *et al.*, 1997 U.S. Patent NO. 5,605,671. For example, labeled TSG-5 chemokine could be utilized to image sites of infection, inflammation, neoplasm, restenosis, atheromatous lesions or sites undergoing other immune reactions. The preferred imaging agents would include radioactive isotopes of indium, iodine, technetium, rhenium, gallium, samarium, holmium, yttrium, copper, cobalt and the like. Other contemplated labelling methods include fluorescent labelling, metallic labelling and/or chelate labelling. Labelled antibodies, such as radio labelled antibodies, for use in imaging are also contemplated. Particularly preferred embodiments would use a radioactive label selected from the group: ^{99m}Technitium, ^{111}Indium, ^{62}Copper, ^{123}Iodine, ^{131}Iodine, ^{186}Rhenium and ^{188}Rhenium. Another embodiment contemplated would use ^{13}carbon as the labeling agent to determine sites of TSG-5, TSG-5v1 or TSG-5v2 activity by magnetic resonance spectroscopy (MRI). Methods of making the labeled such protein or peptides and using them *in vivo* would be known to the skilled artisan and as taught, for example, by Lyle *et al.* 1997.

All references as discussed in this specification are herein incorporated by reference.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

14. Diagnostic and Therapeutic Uses of TSG-5, TSG-v1 or TSG-5v2

As discussed above, TNF-α stimulated genes and proteins are associated with inflammatory states and disorders. Consequently, the presence or expression of TNF-α stimulated genes (i.e., "TSGs") can be an effective diagnostic tool. It is a contemplated method of the invention that inflammation associated disorders can be diagnosed by the

detection of increased levels of TSG-5, TSG-5v1 and TSG-5v2 polypeptides or nucleic acid expression in a cell.

It is further noted that determining the levels of TSG-5, TSG-5v1 or TSG-5v2 can provide an assay of the responsive effects of TNF-α on a cell. Modulating TSG-5, TSG-5v1 and TSG-5v2 levels can provide a method of involving the effedcts of TNF-α on a cell. Detection may be accomplished by any of several means known in the art; ELISA or PCR techniques are preferred.

TSGs also form part of the body's natural responses to inflammation producing diseases. Consequently, it is contemplated that administration of therapeutically attestive amounts of TSG-5, TSG-5v1 and TSG-5v2 can assist or supplement natural response to inflammation. Methods of administration of TSG-5, TSG-5v1 or TSG-5v2 are as described for agents and antibodies of the invention described above.

EXAMPLES

Example 1

Isolation and Identification of TSG-5

TNF was originally described as a factor responsible for the necrotic reaction observed in some induced tumor models. Once isolated, TNF proved to be a pro-inflammatory cytokine with pleiotropic activities (Feinman *et al.*, 1987 J. Immunol. 138: 635-640; Lin *et al.*, 1987 J. Biol. Chem. 262: 11908-11911; Palombella *et al.*, 1988 J. Cell. Physiol. 135: 23-31; Probert *et al.*, 1997 J. Neuroimmunol. 72: 113-117; Schutze *et al.*, 1992 Semin. Oncol. 19: 16-24; Tisdale, 1997 J. Nat'l Cancer Inst. 89: 1763-1773; Vilcek *et al.*, 1988 Ann. Inst. Pasteur Immunol. 139: 307-311; Vilcek *et al.*, 1991 J. Biol. Chem. 266: 7313-7316; and Vilcek *et al.*, 1992 Immunol. Ser. 87: 269-287). In order to characterize the mechanism of action of TNF, we used a differential display approach to compare the mRNA content of cells treated with TNFα to untreated cells to identify new TNFα-inducible genes, of which TSG-5 is one.

Materials and Methods. cDNA Synthesis. Mouse embryonic fibroblasts (MEFs) derived from either wild-type or IRF-1 deficient animals (Reis *et al.*, 1994) were treated with recombinant TNFα (Genzyme 30 U/ml) for 4 hr or left untreated (control). Total RNA was extracted and treated with DNAse I for 30 minutes. 200 ng of DNA-free total RNA was reverse transcribed with MMTV-RTase using an anchored oligonucleotide (T_{11}VA, T_{11}VC, T_{11}VT, T_{11}VG, where V can be A, C or G) as primer. From the RTase reaction, 2 µl of the cDNA were used as a template in the PCR reaction using the same anchored PCR primer, plus a random primer 10 nucleotides long, in the presence of ^{32}P-adCTP.

Northern Blot analysis. The original total RNA from TNFα- treated IRF-1o/o MEFs was probed with the TSG-5 fragment of SEQ ID No. 7, revealing a band of approximately 600-700 bp. This band was identified mainly in TNFα-treated IRF-1 deficient cells (Figure 1A) and corresponds to the nucleic acid in SEQ ID NO. 7. TSG-5 was inducible by TNF in control, wild-type cells as revealed by RT-PCR Figure 1B.

Genomic DNA Analysis. The TSG-5 fragment was used to screen a mouse genomic library and two independent phages were identified and isolated. One phage had an insert corresponding to a 15 kb mouse genomic DNA that contained sequences which hybridized with the TSG-5 fragment. Purified phage DNA was digested with different restriction enzymes, as indicated. Restriction analysis and subsequent hybridization revealed the presence of several bands that hybridized with the TSG-5 probe (Figure 2). The digested DNA was electrophoresed through a 0.8% gel. The fragments were identified by hybridization using a radioactively labeled TSG-5 probe.

The digested DNA fragments, indicated by the arrowheads, were subcloned and sequenced. The *HindIII* fragment was sequenced in both orientations and revealed the presence of the originally identified TSG-5 sequence (Figure 3; SEQ ID NO. 1).

The entire TSG-5 sequence generated from the genomic *HindIII* fragment is set forth in SEQ ID NO. 3. Additional sequencing of genomic DNA clones provided the sequence of SEQ ID NO. 4. Mouse genomic DNA was isolated from tail biopsies and digested with different restriction enzymes, as indicated. The digested DNA was electrophoresed through a 0.8% agarose gel, and the fragments containing the TSG-5 gene were identified by hybridization using radioactively labeled TSG-5 probe.

Results. From the several differentially displayed bands, one band was cut, eluted from the gel, cloned and sequenced (SEQ ID NO. 7). This band, named 059A14+, TSG-5, was obtained with the primers $T_{11}VC$ (SEQ ID NO. 10) and the random primer CGTCTCTCAA. The sequence obtained was compared with known sequences deposited on different databases using BlastN (Altschul *et al.*, 1997 Nuc. Acids Res. 25:3389-3402). No significant homologues were identified corresponding to TSG-5. The sequence of GenBank Accession No. AF042005 corresponds to the TSG-5 genomic sequence later isolated using the 059A14+ cDNA (SEQ ID NO. 3).

The genomic sequence has an Open Reading Frame which could encode an 113 amino acid protein as depicted in Figure 4 (SEQ ID NO. 1) and SEQ ID No. 2. The start codon for this protein forms a good Kozak consensus sequence according to the program NetStar 1.0 (available at: www.cbs.dtu.dk/services/netstar by Pedersen *et al.*, 1997) as indicated in Figure 3 (SEQ ID NO. 1). The protein program PROPSEARCH (Hobohm *et al.*, 1995 J. Mol. Biol. 251: 390-399) was used and identified a similarity between TSG-5 and the CC family of chemokines, with a reliability of 53%. An alignment between TSG-5 and MIP-3b is shown in Figure 4. TSG-5 has a putative signal peptide that provides a cleavage site between amino acid residues 16 and 17, has two adjacent cysteines (hence a member of the CC family of chemokine), and the molecular weight of 12.9 kD. The TSG-5 protein also contains a potential myristylation site. Such a translational modification of TSG-5 is uncharacteristic of chemokines and may indicate that TSG-5 is anchored to the inner face of the cell membrane. Again, when the sequence displayed in SEQ ID NO. 3 or 4 was searched using BlastN; no significant homologues were identified.

TSG-5 is a single copy gene and appears to lack introns. It should be noted, however, that alternatively spliced transcripts have been identified (See Example 3 below). The non-coding sequences upstream from the start codon contains putative binding sites for several transcription factors (TFs) associated with pro-inflammatory cytokines (see Figure 7; SEQ ID NO. 8). These TFs are particularly active in immune competent cells, such as macrophages, fibroblasts and lymphocytes

Example 2

Expression and Analysis of Recombinant TSG-5 Protein

Materials and Methods. The genomic sequence corresponding to the TSG-5 Open Reading Frame (SEQ ID NO. 1) was cloned and expressed in *E.coli* using the His-Tag approach for its purification. Purified protein showed no contamination by LPS using the LAL assay (Limulus Amebocyte Lysate KCL-1000, from BioWhittaker, Walkerville, MD). To test chemotactic properties of TSG-5, rat eosinophils were isolated from the peritoneal cavity and distributed ($9x10^5$ in 100ml) in the upper chamber of a 24-well Transwell plate with pore size of 5.0μm (Corning Coastar Corp., Cambridge, MA). *E.coli*-derived TSG-5 or Eotaxin was added to the bottom chamber at the indicated concentrations, diluted in Hank's medium. The number of migrating eosinophils was evaluated by microscopy.

Results. Recombinant TSG-5 protein was assayed for chemotatic activity using Rat eosinophils. As shown in Figure 10, TSG-5 induced a significant chemotatic activity, with a chemotaxis index of six, corresponding to a six-fold increase in the number of migrating eosinophils in the presence of TSG-5 as compared to the negative control (medium alone). Eotaxin, the best characterized eosinophil-specific CC chemokine showed a chemotaxis index of 3.

Example 3
Characterization of other TSG-5 DNAs:

A mouse cDNA library was prepared from PolyA+ mRNA derived from mouse embryonic fibroblasts that were stimulated with TNF for 8h and screened with the nucleic acid of SEQ ID NO. 7. Six cDNA clones having at least partial homology to the TSG-5 genomic sequence (SEQ ID NO. 4) were identified. Sequences from two of these clones, TSG-5v1 (SEQ ID NO. 13) and TSG-5v2 (SEQ ID NO. 15), and their predicted amino acid sequences are presented (SEQ ID NOS. 14 and 16, respectively). The presence of a nuclear localization sequence ('RRKR') in the predicted protein sequences suggests that these proteins are nuclear protein proteins.

Comparison of the sequences of these clones with the active, recombinant protein is shown in Figure 11 and suggests alternative splicing and translation pathways for this gene. For example, nucleotides 51 to 62 of SEQ ID NO. 13 are not present in SEQ ID NO. 15, suggesting the presence of a differentially spliced exon. Also of note is the observation that the identified cDNAs terminate 5' to the stop codon of TSG-5. The resulting ORFs for TSG-5v1 and TSG-5v2 are based on the next available stop codon which is out of frame with the predicted sequence for TSG-5.

The presence of the transcripts encoding the "recombinant" TSG-5 protein in MEF RNA samples, in addition to the transcripts corresponding to SEQ ID NOS. 13 and 15, is supported by fact that cDNAs have been generated from isolated RNA that is devoid of genomic DNA contamination using primers within the genomic sequence and that would anneal to sequences present in TSG-5 (SEQ ID No. 1), but that are absent from the TSG-5v1 (SEQ ID No. 13) and TSG-5v2 (SEQ ID No. 15). It is further noteworthy that the recombinant TSG-5 protein predicted from the genomic sequence functions as a chemokine, and that antibodies against the recombinant TSG-5 recognize a protein that is expressed in liver and lung of both untreated and LPS-treated mice, with higher reactivity in the latter.

Example 4

Identification of Agents which Induce TSG-5

Materials and Methods. For the preparation of data shown in Figure 6, mice were injected *i.p.* with thioglycolate. Peritoneal macrophages were harvested 3 days later. Macrophages were washed twice with cold PBS and induced with TNFα (30 ng/ml), LPS (0.1 μg/ml). IFNα (100 U/ml) or LPS plus IFNγ for 12 h. Total RNA was extracted, treated with DNAse. 1 μg of DNA-free total RNA was reverse transcribed (MMTV-RTase) using the TSG-5 specific primer. From the PCR reaction, 2 μl were used for PCR using the Primers 5' CAAAATGCAGGGAATGAATG3' (forward) (SEQ ID No. 11) and 5' AGAGAGCCTGGGTTTGGTT 3' (reverse) (SEQ ID No. 12). The PCR product was resolved on a 1% agarose gel. Southern blot analysis was performed using the 240 bp fragment of the TSG-5 gene as a radioactively labeled probe.

Results. Figure 6 demonstrates that TNFα, IFNα and LPS can all induce expression of TSG-5 in different cell types. Other cytokines, such as TNFβ and IFNα may also

modulate the activity of TSG-5. Putative binding sites related to TNF and IFN signaling are underlined in Figure 7, which depicts the TSG-5 promoter (SEQ ID No. 8).

Example 5

Method of Isolating Human TSG-5

Materials and Methods. Human TSG-5, as well as genes encoding TSG-5 in other species, can be isolated by utilizing the murine TSG-5 nucleic acid molecule to hybridize to human cDNAs from an appropriate human library (*e.g.*, human macrophage cDNA library). Alternatively, one skilled in the art could utilize genomic DNA (from the desired species) and digest it with restriction enzymes recognizing hexamers (*e.g.*, *Bam HI, Xba I, Bgl II, Eco RI, etc.*). The digested DNA is separated in a 0.8% agarose gel. After transfer of the separated DNAs to a nylon filter, Southern blot analysis is performed using low stringency hybridization conditions and washing the filter using wash solutions to increase the stringency conditions, optimizing signal to noise ratio. Stringent hybridization conditions are determined according to the membrane used as discussed in Sambrook *et al.*, (1989).

Additionally, degenerate primers to either the coding or non-coding sequences of the murine TSG-5 can be used to isolate the human TSG-5 homolog. Degenerate primers can be synthesized that correspond to the sequences encoding the amino and carboxy terminal residues of murine TSG-5. Alternatively, degenerate primers could be designed to the sequences immediately upstream and down stream of the conserved CC motif (the nucleotide sequences corresponding to ^{34}TKDQGIC^{40} and antisense to the nucleotide sequence encoding ^{60}CVSVGH^{65} of Figure 4; SEQ ID NO. 2). Degenerate primers encoding a different conserved residue (*e.g.*, ^{35}K→R or ^{36}D→E) can be introduced into these primers. Degenerate primer length can range in size from 16-30 nucleotides, with the number of nucleotide changes introduced into the primer dependent on length, as discussed *infra*.

Alternatively, degenerate primers could be synthesized to non-coding regions of the *TSG-5* gene. Such primers could include nucleotide mutations in regions of TSG-5 gene not now known to certain transcriptional or translational information. Alternatively silent mutations can be incorporated into the coding region of the *TSG-5* gene without inducing changes to the amino acid sequence.

Using the foregoing strategy, southern blot analysis of human genomic DNA has suggested the presence of a sequence that appears to share some degree of homology to the murine TSG-5.

Example 6

Preparation of Antibodies which recognize TSG-5

Materials and Methods. Animals, such as rabbits, can be injected with about 0.2 mg of TSG-5 emulsified in Freund's complete adjuvant. The animal is then boosted on an injection schedule suitable to that animal using Freund's incomplete adjuvant. Polyclonal sera containing anti-TSG-5 antibodies can then be obtained.

For Figure 8A, the spleens from control or LPS-treated mice (50 μg/animal; *i.p.* for 18 hours) were collected and single cell suspensions were prepared. For flow cytometry, cells were permeabilized and stained with a rabbit anti-TSG-5 antibody prepared against the RNHQARGEEEKGLATKDQGIC peptide (SEQ ID No. 9). The antigenicity of this peptide was predicted from the ORF of the genomic TSG-5 sequence. A FITC-labeled goat anti-rabbit antibody was used as a secondary antibody.

For Figure 8B, thymuses from control or LPS-treated mice (50 μg/animal, *i.p.* for 18 hours) were collected and single cell suspensions were prepared. For flow cytometry, cells were permeabilized and stained with a rabbit anti-TSG-5 antibody prepared against the RNHQARGEEEKGLATKDQGIC peptide (SEQ ID No. 9). A FITC-labeled goat anti-rabbit antibody was used as a secondary antibody.

Results. As shown in Figure 8A-B, TSG-5 is inducible in thymocytes exposed to LPS. TSG-5 is also expressed in splenocytes.

Example 7

Preparation of anti-TSG-5 Monoclonal Antibody

Materials and Methods. Recombinant TSG-5 protein (*e.g.*, 10-20 μg of the peptide fragment used to prepare the polyclonal antibody in Example 4 or the complete TSG-5 protein) is emulsified in Complete Freund's Adjuvant. The emulsified suspension is injected into a mouse for every 7 to 10 days for a total of 4 to 5 immunization boosts. For a perfusion

boost, the mouse is injected with TSG-5 in PBS, and four days later the mouse is sacrificed and its spleen removed. The spleen is placed in 10 ml serum-free RPMI 1640, and a single cell suspension is formed by grinding the spleen between the frosted ends of two glass microscope slides submerged in serum-free RPMI 1640, supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 1,000 U/ml penicillin and 100 µg/ml streptomycin. The cell suspension is filtered through a sterile 70 mesh Nitex cell strainer (Becton Dickinson) and is washed twice by centrifuging at 200 g for 5 min. The pellet is resuspended in 20 ml serum-free RPMI. Thymocytes taken from three naive BALB/c mice are prepared in a similar manner and used as a control. NS-1 myeloma cells, kept in log phase in RPMI with 11% fetal bovine serum (FBS) for three days prior to fusion, are centrifuged at 200 x g for 5 min. The pellet is washed twice as described previously.

$1x10^8$ spleen cells are combined with $2.0x10^7$ NS-1 cells and centrifuged. After removing the supernatant, the cell pellet is dislodged by tapping the tube and 1 ml of PEG 1500 (50% in 75 mM HEPES, pH = 8.0) at 37°C is added with stirring over the course of 1 minute followed by the addition of 7 ml of serum free RPMI over 7 min. An additional 8 ml RPMI is added and the cells are centrifuged at 200 g for 10 min. After discarding the supernatant the pellet is resuspended in 200 ml RPMI containing 15% FBS, 100 µM sodium hypoxanthine, 0.4 µM aminopterin, 16 µM thymidine (HAT), 25 U/ml IL-6 and $1.5x10^6$ thymocytes/ml and plated onto flat-bottom 96 well tissue culture plates.

On days 2, 4 and 6 after the fusion, 100 µl of medium is removed from the wells of the fusion plates and replaced with fresh medium. On day 8, the fusion is screened by ELISA, testing for the presence of mouse IgG binding to TSG-5. Such screening would be as described in E. Harlow, ANTIBODIES: A LABORATORY MANUAL (1988) and in Godiska *et al.*, U.S. Patent 5,688,927 (1997).

Example 8

Tissue Expression of TSG-5

Tissue specific expression of TSG-5 can be ascertained either at the level of mRNA or protein synthesis. TSG-5 protein expression (either *de novo* synthesis or steady-state concentrations) can be ascertained using antibodies which recognize TSG-5 protein in

immunoprecipitation experiments or Western blot assays. Methods of preparing TSG-5 proteins are described above and are known in the art, or as described in E. HARLOW, ANTIBODIES: A LABORATORY MANUAL. (Cold Spring Harbor Press, New York 1988).

RNA obtained from the various animal tissues can be obtained using normal methods. The RNA can be separated using electrophoresis and subjected to Northern blot analysis. The blot can be hybridized with labeled DNA encoding TSG-5. If obtaining RNA from animals such as mice, the mice can be administered with TNFα, LPS or IFN. After an appropriate treatment with these agents, the mice are sacrificed and tissues harvested as above. Identification of tissues in which TSG-5 mRNA or protein synthesis is upregulated can then be determined.

Example 9

TSG-5 *In Vivo* Tumor Growth Inhibition Assay

Materials and Methods. Tumor growth-inhibition properties of TSG-5 can be assayed by modifying the protocol described by Laning *et al.*, 1994 J. Immunol. 153: 4625-4635 for assaying the tumor growth-inhibitory properties of murine TCA3. A TSG-5-encoding cDNA is transfected by electroporation into a myeloma-derived cell line (*e.g.*, J558 of the American Type Culture Collection of Manassas, Virginia). Transfectants are screened for TSG-5 production by standard techniques, such as ELISA (enzyme linked immunosorbant assay) using a monoclonal antibody generated against TSG-5. A bolus of about 10 million cells from a TSG-5 producing clone is injected subcutaneously into the lower right quadrant of a BALB/c mouse. For comparison, about 10 million non-transfected cells are injected into a control mouse. The rate and frequency of tumor formation in the two groups is compared to determine the rate and frequency of tumor formation in the two groups. The nature of the cellular infiltrate subsequently associated with the tumor cells is identified by histologic means. In addition, recombinant TSG-5 (about 20 ng) is mixed with non-transfected J558 cells and injected (20 ng/day) into tumors derived from such cells to assay the effect of TSG administered exogenously into tumor cells. Similar experiments can be carried out using other tumor lines to determine the inhibitory effects of TSG-5 on the other malignancies.

Another model system which can be utilized is the "air pouch" model described by Wisniewski *et al.*, 1996 J. Immunol. 156: 1609-1615. As shown by these authors, human recombinant TSG-6 protein demonstrated a potent anti-inflammatory activity in the murine air pouch model of carrageenan- or IL-1-induced cellular infiltration that was comparable to that of systemic dexamethasone treatment. The treatment protocol used with TSG-6 could also be used to evaluate TSG-5.

Example 10

Nucleic Acids which Hybridize to TSG-5

Materials and Methods. To identify nucleic acid molecules which hybridize to the murine TSG-5 nucleotide sequence set forth in Figure 4 (SEQ ID No. 1) or SEQ ID NOS. 3, 4, 13, or 15, hybridization assays are performed using any available methods to control the stringency of hybridization. Hybridization is a function of sequence identify (homology), G+C content of the sequence, buffer salt content, sequence length and duplex melt temperature (T[m]) among other variables. See Maniatis *et al.* (1982) and Sambrook *et al.*, (1989).

Hybridization analysis is performed using genomic DNA or cDNA pools prepared from mRNA from a lymphoid or myeloid cell line according to the procedures of Sambrook *et al.* Chapter 9 (1989). Briefly, hybridization with nylon membranes is performed in 6 X SSC (or 6 X SSPE); 0.5% SDS; 100 µg/ml denatured, sonicated salmon sperm DNA and 50% formamide at 42°C using radiolabeled probe comprising a sequence of either of Figures 4 or SEQ ID NOS. 1, 3 or 4. After hybridization, the filter is washed in 2 X SSC and 0.1% SDS, followed by washes in 0.1 X SSC and 0.5% SDS at 37°C and 0.1 X SSC and 0.5% SDS at 68°C. Results are visualized by autoradiography.

For example, nucleic acid molecules comprising the following sequences hybridize to probe comprising of a sequence in SEQ ID NO. 3 under the above conditions.

gggaatgaag ggggagccca gtggctatta

1441 ccacaccctc aaaacgactc ctgtgccaaa ggatcagaaa tcatcaagca agaggggaag

1501 aagaaaaagg gttagcaacg aaggaccagg gaatctgctg tgagagtgtg tctcctgaac

1561 tgattattaa agatttattt ttatgtgtat ggatatgtgt gtatgtgcag catttatatg

1621 cagtgcttac agaggctaga agagcatatc agatcccctg gacctggagg tacagacagt
1681 tatgaagaccc catgaaagtg ctaggaacca aacccaggct ctctgcaaag caaccactga
1741 gctttctctc ctttatttaa [SEQ ID No. 5]

gggaatgaag ggggagccca gtggctatta
1441 ccacaccctc aaaacgactc ctgtgccaaa ggatcagaaa tcatcaagca agaggggaag
1501 aagaaaaagg gttagcaacg aaggaccagg gaatctgctg tgagagtgtg tctcctgaac
1561 tgattattaa agatttattt ttatgtgtat ggatatgtgt gtctgtgcag catttatatg
1621 cagtgcttac agaggctaga agagcatatc agatcccctg gacctggagg tacagacagt
1681 tattagaccc catgaaagtg ctaggaacca aacccaggct ctctgcaaag caaccactga
1741 gctttctctc ctttatttaa [SEQ ID No. 6]

In SEQ ID No. 5 there are 5 single nucleotide changes all which result in silent mutations in the coding region of SEQ ID NO.3. In SEQ ID No. 6, there are 3 single nucleotide changes which also would yield silent mutations. The start codon is indicated by the underlined "atg" triplet. The putative mutations are indicated in bold and double underlined. Other examples of DNAs which would hybridize with the nucleic acid of SEQ ID NO. 3, or by extension SEQ ID No. 4 would be obvious to the skilled artisan.

Example 11

Western Blot Detection of a TSG-5 Protein in MEFs Exposed to TNFα

Materials and Methods. One method of detecting the presence of TSG-5 in cells or serum is by immune reactions such as immunoprecipitation or Western blotting. In this experiment, mouse embryonic fibroblasts (MEFs) were treated with TNFα for 4 hr. As indicated, cells were pre-treated with 20 μM sodium salicylate (NaSa) for 30, 60 or 120 min followed by treatment with TNFα. Proteins were extracted using a RNAzol procedure. Proteins were extracted, separated on an 8% PAGE, transferred to a nitrocellulose filter and probed using the anti-TSG-5 antibody described in Figure 8. For detection, a secondary, horseradish peroxidase (HRP)-labeled goat and rabbit IgG was also utilized.

Results. Figure 9 depicts two bands that were detected by the anti-TSG-5 antibody. The low molecular weight band migrates in the range of a 45 to 60 kD protein. This slower migrating protein is believed to be a protein complex containing TSG-5.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents and publications referred to in this application are herein incorporated by reference in their entirety.

CLAIMS

We claim:

1. An isolated nucleic acid molecule encoding a TSG-5, TSG-5v1 or TSG-5v2 protein, a variant of TSG-5, TSG-5v1 or TSG-5v2, or a fragment of TSG-5, TSG-5v1 or TSG-5v2.

2. The nucleic acid molecule of claim 1 wherein the nucleic acid encodes a TSG-5 protein, a variant of TSG-5 or a fragment of TSG-5.

3. The nucleic acid molecule of claim 1 wherein the nucleic acid encodes a TSG-5v1 or TSG-5v2 protein, a variant of TSG-5v1 or TSG-5v2, or a fragment of TSG-5v1 or TSG-5v2.

4. A nucleic acid molecule encoding a TSG-5, TSG-5v1 or TSG-5v2 protein which hybridizes under stringent conditions to a nucleic acid comprising nucleotides 80 to 418 of SEQ ID NO. 1, nucleotides 11 to 280 of SEQ ID NO. 13, or nucleotides 2 to 277 of SEQ ID NO. 15.

5. A nucleic acid molecule encoding a TSG-5 protein which hybridizes under stringent conditions to a nucleic acid comprising nucleotides 80 to 418 of SEQ ID NO. 1.

6. A nucleic acid molecule encoding a TSG-5v1 or TSG-5v2 protein which hybridizes under stringent conditions to a nucleic acid comprising nucleotides 11 to 280 of SEQ ID NO. 13, or nucleotides 2 to 277 of SEQ ID NO. 15.

7. The nucleic acid molecule of claim 1, 2 or 3, wherein the nucleic acid molecule encodes a mammalian protein.

8. The nucleic acid molecule of claim 4, 5 or 6, wherein the nucleic acid molecule encodes a mammalian protein.

9. The nucleic acid molecule of claim 1, 2 or 3, wherein the nucleic acid molecule encodes a human protein.

10. The nucleic acid molecule of claim 4, 5 or 6, wherein the nucleic acid molecule encodes a human protein.

11. An expression vector comprising the nucleic acid molecule of any one of claims 1, 2, 3, 4, 5 or 6.

12. The expression vector of claim 11 wherein the encoded protein is mammalian.

13. The expression vector of claim 11 wherein the encoded protein is human.

14. A host cell transformed with the expression vector of claim 11.

15. A method for producing a TSG-5, TSG-5v1 or TSG-v2 protein, a variant of said protein or a polypeptide fragment of said protein comprising the steps of:

culturing a host cell of claim 12 under conditions suitable for the expression of said protein, said variant or said polypeptide fragment; and

purifying said protein, said variant or said polypeptide fragment from the host cell.

16. An isolated polypeptide comprising the amino acid sequence of a mammalian TSG-5, TSG-5v1 or TSG-5v2 protein, wherein said protein is encoded by the nucleic acid molecule of claim 1, 2, 3, 4, 5 or 6.

17. An isolated polypeptide comprising the amino acid sequence of a mammalian TSG-5, TSG-5v1 or TSG-5v2 protein, wherein said protein is encoded by a nucleic acid molecule which hybridizes under stringent conditions with a probe having the nucleotide sequence of the complement of the coding sequence encoding the protein of SEQ ID NOS. 2, 14 or 16.

18. An antibody or antibody fragment which binds to a the protein, the variant or the polypeptide fragment of claim 16.

19. An antibody or antibody fragment which binds to a the protein, the variant or the polypeptide fragment of claim 17.

20. The antibody of claim 18, wherein the antibody is a monoclonal antibody.

21. The antibody fragment of claim 18, wherein the antibody fragment is selected from the group consisting of scFv, Fab, Fab' and $F(ab')_2$.

22. The antibody of claim 20 or 21 wherein the antibody is chimeric or humanized.

23. The antibody of claim 20 or 21 wherein the antibody is chimeric or humanized.

24. A method of imaging a target site containing TSG-5, TSG-5v1 or TSG-5v2 in a subject, comprising the steps of:

providing a labeled TSG-5, TSG-5v1 or TSG-5v2 polypeptide;

introducing the labeled polypeptide into the subject; and

detecting the labeled polypeptide thereby imaging the target site.

25. The method of claim 24, wherein the target site is a tissue undergoing a TSG-5, TSG-5v1 or TSG-5v2 mediated activity.

26. The method of claim 24, wherein said peptide is labeled with a radiolabel.

27. The method of claim 19 wherein said peptide is labelled with a flourescent, metallic or chelate label.

28. The method of claim 26, wherein said radiolabel is selected from the group consisting of ^{99m}Technitium, ^{111}Indium, ^{62}Copper, ^{123}Iodine, ^{131}Iodine, ^{186}Rhenium and ^{188}Rhenium.

29. A method of imaging a target site containing TSG-5, TSG-5v1 or TSG-5v2 in a subject, comprising the steps of:

providing a labeled TSG-5, TSG-5v1 or TSG-5v2 antibody;

introducing the labeled antibody into the subject; and

detecting the labeled antibody thereby imaging the target site.

30. A method of modulating the expression of a nucleic acid encoding the protein having the amino acid sequence of SEQ ID NO. 2, SEQ ID NO. 14 or SEQ ID NO. 16, said method comprising the step of:

administering an effective amount of an agent which modulates the expression of a nucleic acid encoding the protein having said amino acid sequence.

31. Method of screen for agents that modulate the expression of a nucleic acid encoding a protein having an amino acid sequence comprising the sequence of SEQ ID NO. 2, SEQ ID NO. 14 of SEQ ID NO. 16 comprising the steps of:

providing a candidate agent;

administering a predetermined amount of the agent to a cell; and

determining whether there is a change in the level of expression of said nucleic acid.

32. A method of modulating at least one activity of a protein comprising the amino acid sequence of SEQ ID NO. 2, SEQ ID NO. 14 or SEQ ID NO. 16, said method comprising the step of:

administering an effective amount of an agent which modulates at least one activity of a protein comprising said amino acid sequence.

33. Method of screen for agents that modulate the activity of a nucleic acid encoding a protein having an amino acid sequence comprising the sequence of SEQ ID NO. 2, SEQ ID NO. 14 of SEQ ID NO. 16 comprising the steps of:

providing a candidate agent;

administering a predetermined amount of the agent to a cell; and

determining whether there is a change in the level of activity of said protein.

34. Method of screen for agents that modulate the activity of a nucleic acid encoding a protein having an amino acid sequence of SEQ ID NO. 2, SEQ ID NO. 14 of SEQ ID NO. 16 comprising the steps of:

providing a candidate antibody;

administering a predetermined amount of the antibody to a cell; and

determining whether there is a change in the level of activity of said nucleic acid.

35. A method of identifying binding partners for a protein comprising the amino acid sequence of SEQ ID NO. 2, SEQ ID NO. 14 or SEQ ID NO. 16, said method comprising the steps of:

exposing the protein to a potential binding partner; and

determining if the potential binding partner binds to the protein, thereby identifying binding partners for a protein comprising the said amino acid sequence.

36. A method of diagnosing an inflammatory response comprising the step of detecting the presence of TSG-5, TSG-5v1 or TSG-5v2 in a biological sample.

37. A method of modulating an inflammatory response comprising the step of administering an agonist or an antagonist of TSG-5, TSG-5v1 or TSG activity.

38. A method of modulating chemotaxis of immune cells comprising the step of administering an agonist or an antagonist of TSG-5, TSG-5v1 or TSG activity.

39. A method of modulating proliferation of immune cells comprising the step of administering an agonist or an antagonist of TSG-5, TSG-5v1 or TSG activity.

FIG. 1A

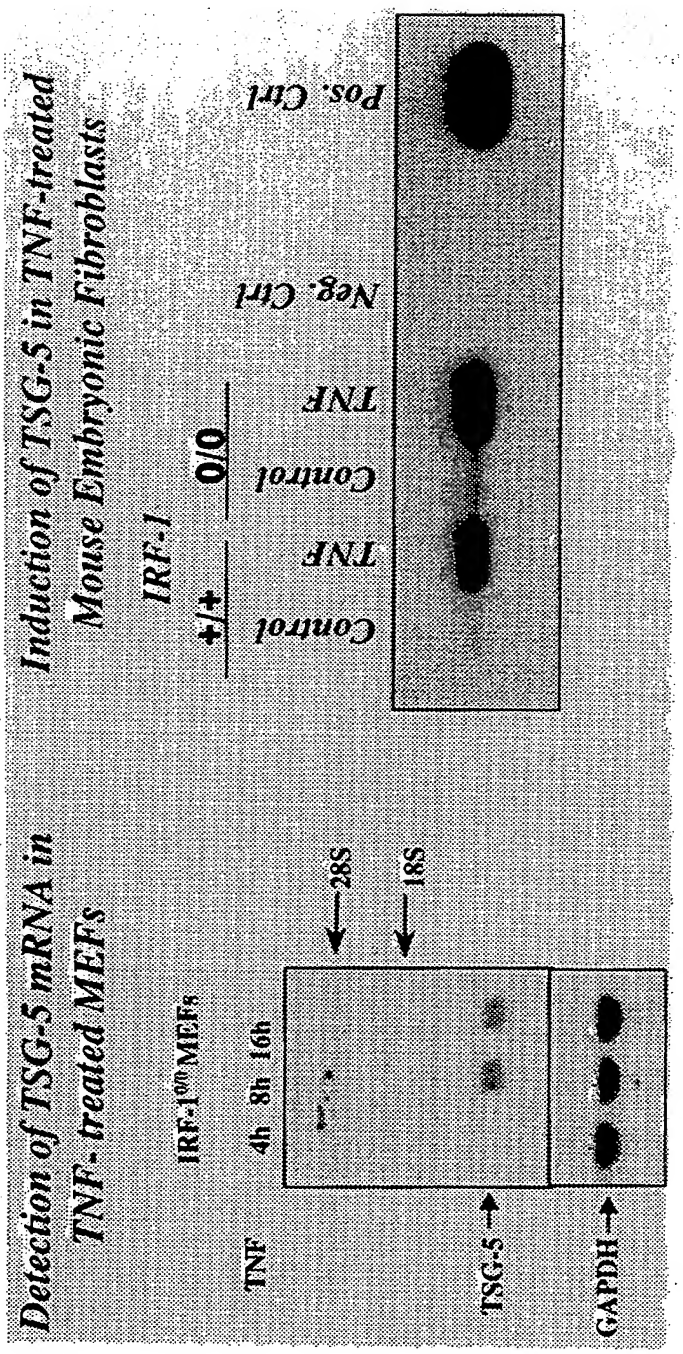

Detection of TSG-5 mRNA in
TNF- treated MEFs
IRF-1(m) MEFs
4h 8h 16h
TNF
28S
18S
TSG-5
GAPDH

FIG. 1B

Induction of TSG-5 in TNF-treated
Mouse Embryonic Fibroblasts
IRF-1
+/+
0/0
Control
TNF
Control
TNF
Neg. Ctrl
Pos. Ctrl

FIG. 2

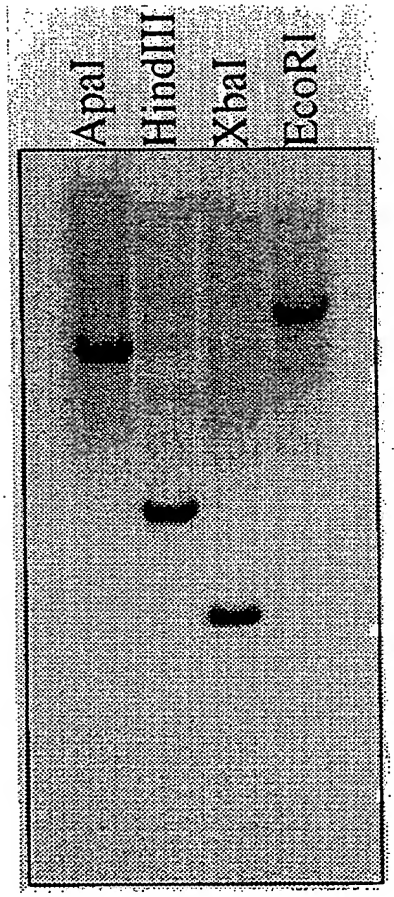


FIG. 3

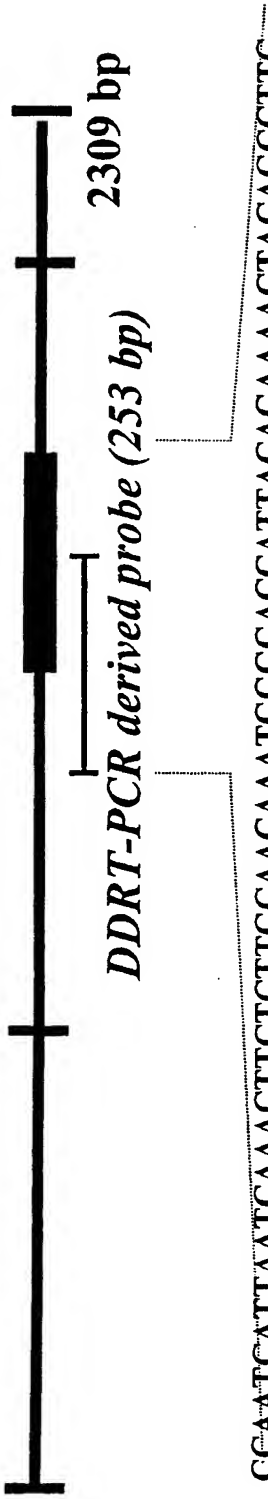

Hind III
Xba I
ORF (113 amino acids)
Xba I
Hind III
2309 bp
DDRT-PCR derived probe (253 bp)
CCAATCATTAATCAAACTTCTCTTGCAACAAATGGGGACCATTACAGAAAACTACAGCCTTG
CAAAATGCAGGGAATGAATGGGGAGCCCAGTGGCTATTACCACACCCTCAAAACAACTCCT
GTGCCAAAGGATCAGAAATCATCAAGCAAGAGGGGAAGAAAAAGGGTTAGCAACGAAG
GACCAGGGAATCTGCTGTGAGATTGTGTCTCCTGAACTGATTATTAAAGATTTATTTTTATG
TGTATGGATATGTGTCTGTGCAGCATTTATATGCAGTGCTTACAGAGGCTAGAAGAGCAT
ATCAGATCCCCCTGGACCTGGAGGTACAGACAGTTATTAGACCCCATGAAAGTGCTAGGAAC
CAAACCCAGGCTCTCTGCAAAGCAACCACTGAGCTTTCTCTCCTCTTATTTAAAAATAAAGAT
GATAGATAAAT
Polyadenylation signal

FIG. 4

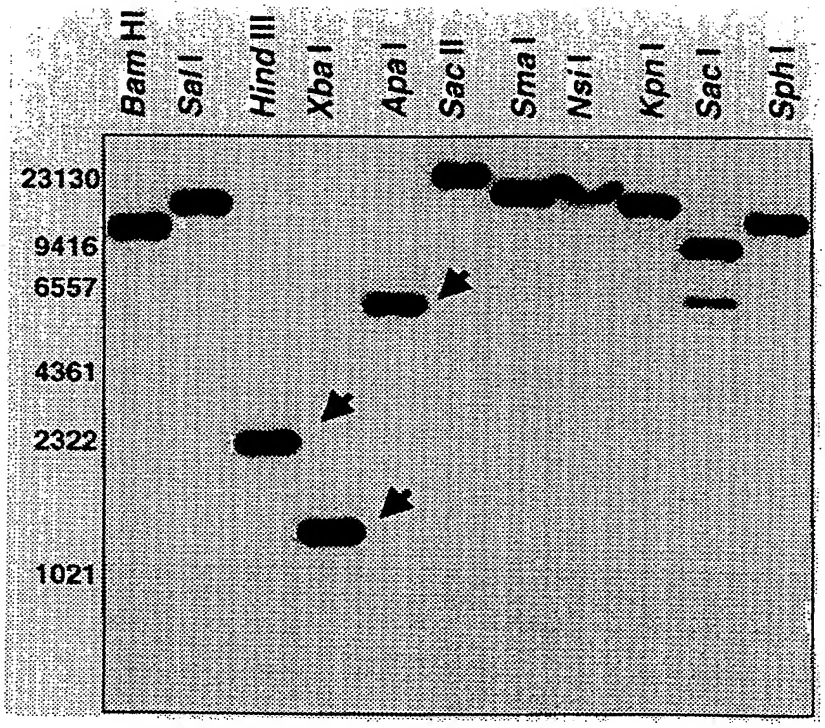
TSG-5

mgspvaittpskgllcgrirnhqargeeekglatkdqgicceivspeliikdlflcvwigvsvqhlyavlteatrayqipwtwryrqlldpmkvlgtkprlsakqplsflsfi

mallalsllvlwtspaptlsgtndaedcclsvtqkpipgyivrnfhyllikdggrvpavvfttlrgrqlcappdqpwveriiqrlqrtsakmkrrss

MIP3-β (Human)

FIG. 5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Sal I
Hind III
Xba I
Apa I
Sac II
Sma I
Nsi I
Kpn I
Sac I
Sph I
23130
9416
6557
4361
2322
1021

FIG. 6

FIG. 7

ggccaatgacccacaagagcttggagacccacatttctawwtgattctgattcatggtttaaaatttgaaatt

Stat-1

aaactcaagtacttttacactctggtcaacctaataaaataaaaattgatttaacaaaa ttctccaaaccacca

Stat-1

ctctgaaaaggrcaaaacattgttgagagaaagaggagaaaaccaaagtargtgaaaatatgacatgcgt

Stat-1

ctbggggttggaaatgtcatcctattagcamaacc ttccccmaattaatctacagatttaagatatgttaaaa

aatattgcaggatgccttttcgagctgatcctaaaatgtacacatacccaaaataaacaaaactatggaaata

avagaaaadgtagaaaggatctcctagttttaaaacctactttaaacctatataaatatgacagtttaaagga

IRF-1/ISRE

cccatcatttaatatatgaggggctctttcagtatgccagtgtatctga gcaaaaagaaagaaacttatgaga

ctgttctgcctactaaaattaaggtagctgctcccacagtgaaaaccttgattcaccactcccaaccagaaaa

Stat-1

ggtcattaaggattcaaagaaacgttgagacattgtttgaatattgccactactgaaattagacaaagaatga

cagggatttgtctagagattctcaagtcatagaatactatcactactcaaattatcttcttacacacttttaaataa

tatagaattatattattcttccctctgccccttcctccctccagtccttcttatatacctcctgccctagctacttct

tg Repeated

caaaattataacctcttctttatt gtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt aaataaac

Stat-1 NF-kB

ttcattagtgtttttagtggttcttgaaagtatctgatttctggggataaccacttggtatttgataatcaatttgggg

NF-kB

ggctcaccccttagggaagactatttctctggctctcagcctctgtaagtttcctatggttctttgaatatggga

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FIG. 8A

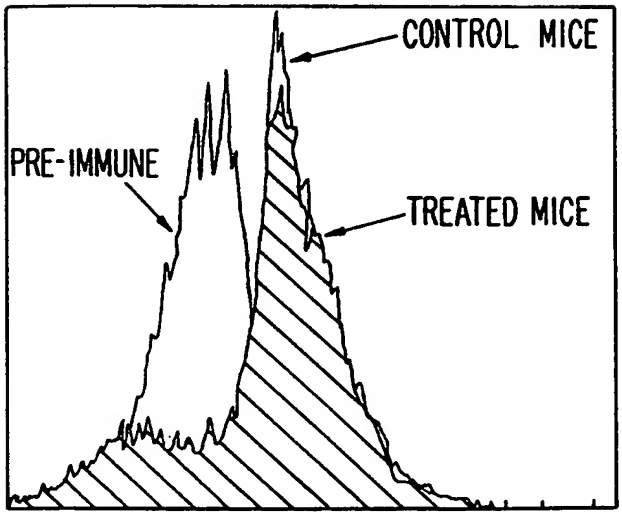

FIG. 8B

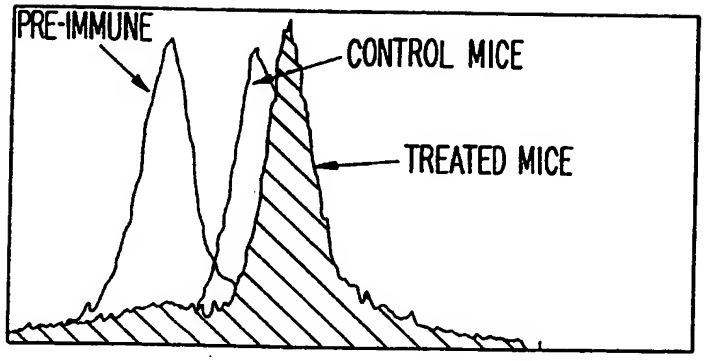

FIG. 9

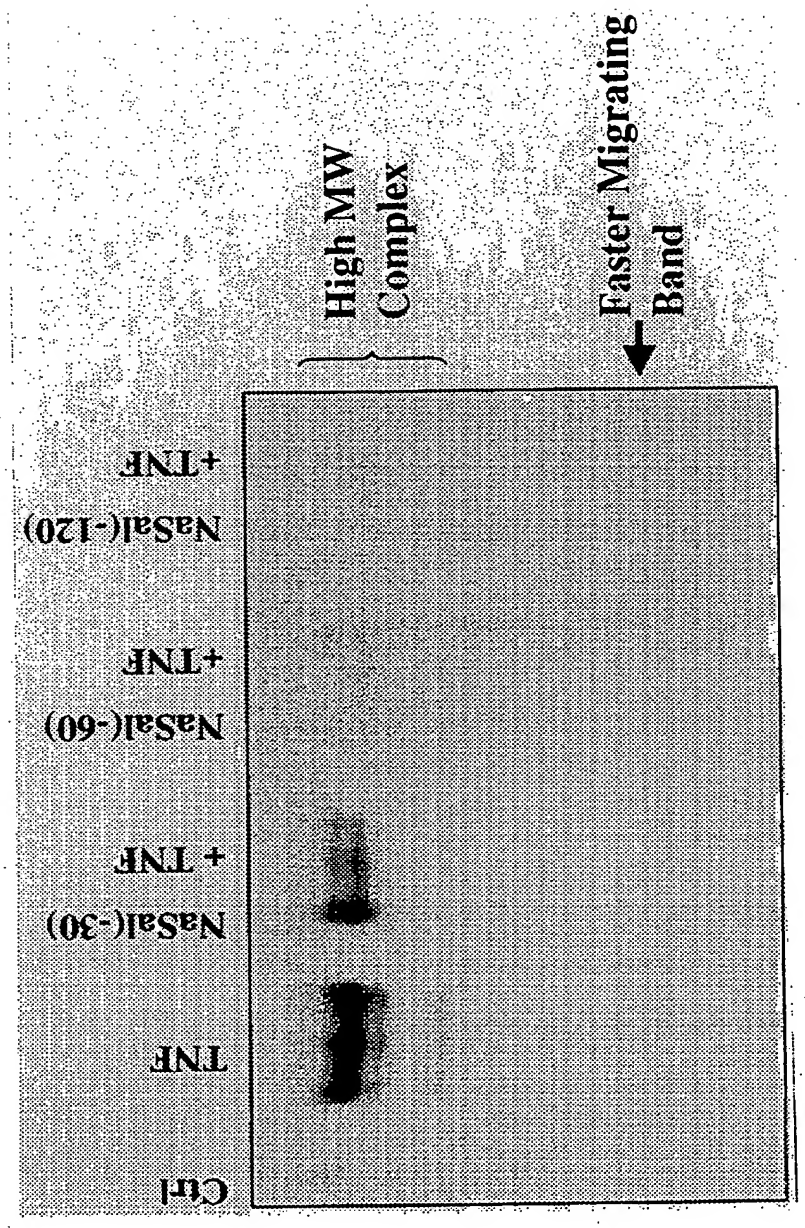
Ctrl
TNF
NaSal(-30)
+ TNF
NaSal(-60)
+TNF
NaSal(-120)
+TNF
High MW
Complex
Faster Migrating
Band

FIG. 10

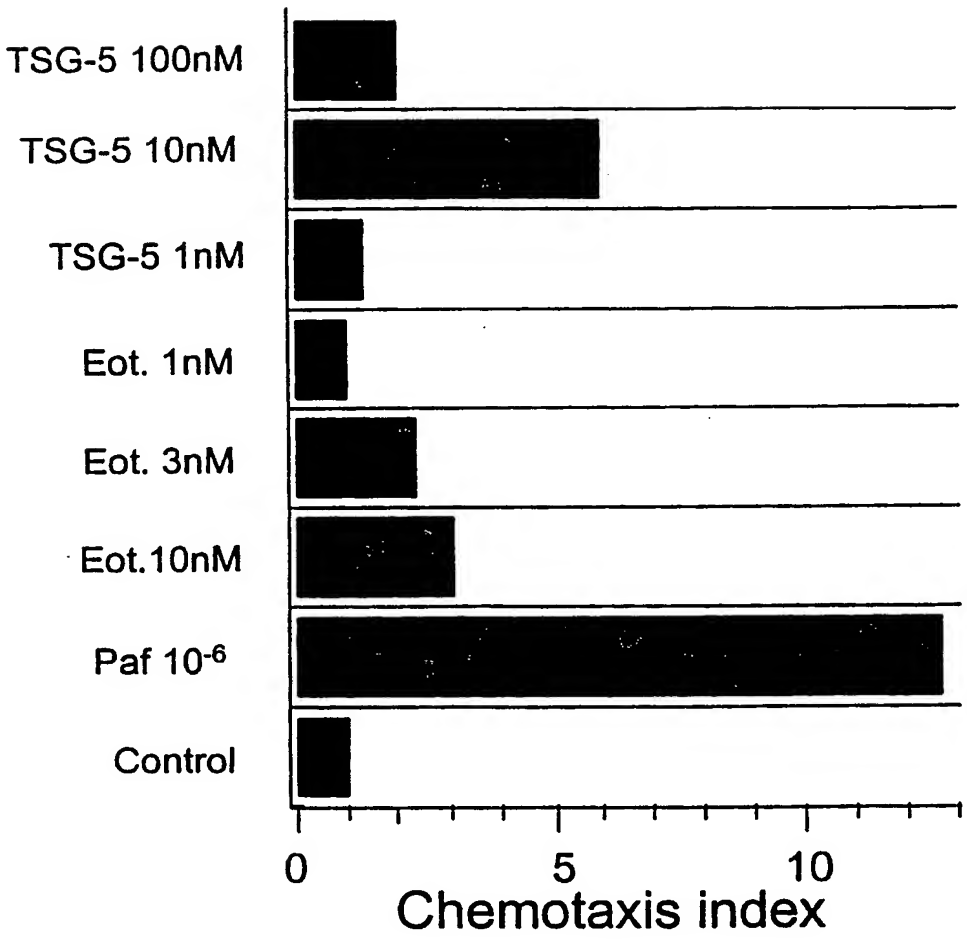
TSG-5 100nM
TSG-5 10nM
TSG-5 1nM
Eot. 1nM
Eot. 3nM
Eot.10nM
Paf 10-6
Control
0
5
10
Chemotaxis index

FIG. 11A

```
TSG-5v2
   TGACCTCTGCATCAGCTCCTGCTTCCTGACCTGCTTGAGTTCCAGTCCTGACTTCCTTG
TSG-5v1
   ..........ATCAGCTCCTGCTTCCTGACCTGCTTGAGTTCCAGTCCTGACTTCCTTG
Genomic sequence
   ............................................................
Clone 059A14+
   ............................................................
TSG-5
   ............................................................


   ............ATAACTATAGCTCTCAACCAATCATTAATCAAACTTCTCTTGCAACA

   GTGATGAACGCAATAACTATAGCTCTCAACCAATCATTAATCAAACTTCTCTTGCAACA

   ............ATAACTATAGCTCTCAACCAATCATTAATCAAACTTCTCTTGCAACA

   ............................CCAATCATTAATCAAACTTCTCTTGCAACA

   ............................................................


   AATGGAGACCATTCACGAAAACTACAGCCTTGCAAAATGCAGGGAATGAATGGGGAGCC

   AATGGAGACCATTCACGAAAACTACAGCCTTGCAAAATGCAGGGAATGAATGGGGAGCC

   AATGGGGACCATTACAGAAAACTACAGCCTTGCAAAATGCAGGGAATGAATGGGGAGCC

   AATGGGGACCATTACAGAAAACTACAGCCTTGCAAAATGCAGGGAATGAATGGGGAGCC

   ..................................................ATGGGGAGCC


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   CAGTGGCTATTACCACACCCTCAAAACAACTCCTGTGCCAAAGGATCAGAAATCATCAA

   CAGTGGCTATTACCACACCCTCAAAACAACTCCTGTGCCAAAGGATCAGAAATCATCAA

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   CAGTGGCTATTACCACACCCTCAAAACAACTCCTGTGCCAAAGGATCAGAAATCATCAA
```

13/14

FIG. 11B

GCAAGAGGGGAAGAAGAAAAAGGGTTAGCAACGAAGGACCAGGGAATCTGCTGTGAGAT

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GCAAGAGGGGAAGAAGAAAAAGGGTTAGCAACGAAGGACCAGGGAATCTGCTGTGAGAT

GCAAGAGGGGAAGAAGAAAAAGGGTTAGCAACGAAGGACCAGGGAATCTGCTGTGAGAT

GCAAGAGGGGAAGAAGAAAAAGGGTTAGCAACGAAGGACCAGGGAATCTGCTGTGAGAT

TGTGTCTCCTGAATTGATTATTAAAGATTTATTTTTATGTGTATGGA.....

TGTGTCTCCTGAATTGATTATTAAAGATTTATTTTTATGTGTATGGA.....

TGTGTCTCCTGAACTGATTATTAAAGATTTATTTTTATGTGTATGGATATGTGTGTCTG

TGTGTCTCCTGAACTGATTATTAAAGATTTATTTTTATGTGTATG.....

TGTGTCTCCTGAACTGATTATTAAAGATTTATTTTTATGTGTATGGATATGTGTGTCTG

.....

.....

TGCAGCATTTATATGCAGTGCTTACAGAGGCTAGAAGAGCATATCAGATCCCCTGGACC

.....

TGCAGCATTTATATGCAGTGCTTACAGAGGCTAGAAGAGCATATCAGATCCCCTGGACC

.....

.....

TGGAGGTACAGACAGTTATTAGACCCCATGAAAGTGCTAGGAACCAAACCCAGGCTCTC

.....

TGGAGGTACAGACAGTTATTAGACCCCATGAAAGTGCTAGGAACCAAACCCAGGCTCTC

FIG. IIC

.....

.....

TGCAAAGCAACCACTGAGCTTTCTCTCCTTTATTTA

.....

TGCAAAGCAACCACTGAGCTTTCTCTCCTTTATTTA

SEQUENCE LISTING

<110> Ludwig Institute for Cancer Research
Reis, Luiz F. L.
Pires, Eduardo G.
Da Silva, Aristobolo M.
Abrantes, Eduardo F.

<120> TSG-5: A Novel TNF-Inducible Gene

<130> 40750-5005-WO

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<222> (80)..(418)
<223> Fragment of TSG-5 gene

<400> 1
ccaatcatta atcaaacttc tcttgcaaca aatggggacc attacagaaa actacagcct 60

tgcaaaatgc agggaatga atg ggg agc cca gtg gct att acc aca ccc tca 112
Met Gly Ser Pro Val Ala Ile Thr Thr Pro Ser
1 5 10

aaa caa ctc ctg tgc caa agg atc aga aat cat caa gca aga ggg gaa 160
Lys Gln Leu Leu Cys Gln Arg Ile Arg Asn His Gln Ala Arg Gly Glu
15 20 25

gaa gaa aaa ggg tta gca acg aag gac cag gga atc tgc tgt gag att 208
Glu Glu Lys Gly Leu Ala Thr Lys Asp Gln Gly Ile Cys Cys Glu Ile
30 35 40

gtg tct cct gaa ctg att att aaa gat tta ttt tta tgt gta tgg ata 256
Val Ser Pro Glu Leu Ile Ile Lys Asp Leu Phe Leu Cys Val Trp Ile
45 50 55

tgt gtg tct gtg cag cat tta tat gca gtg ctt aca gag gct aga aga 304
Cys Val Ser Val Gln His Leu Tyr Ala Val Leu Thr Glu Ala Arg Arg
60 65 70 75

```
gca tat cag atc ccc tgg acc tgg agg tac aga cag tta tta gac ccc   352
Ala Tyr Gln Ile Pro Trp Thr Trp Arg Tyr Arg Gln Leu Leu Asp Pro
                 80                  85                  90

atg aaa gtg cta gga acc aaa ccc agg ctc tct gca aag caa cca ctg   400
Met Lys Val Leu Gly Thr Lys Pro Arg Leu Ser Ala Lys Gln Pro Leu
             95                 100                 105

agc ttt ctc tcc ttt att taaaaataaa gatgatagat aaat               442
Ser Phe Leu Ser Phe Ile
        110
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<210> 2
<211> 113
<212> PRT
<213> Mus musculus

<400> 2

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Met Gly Ser Pro Val Ala Ile Thr Thr Pro Ser Lys Gln Leu Leu Cys
  1               5                  10                  15

Gln Arg Ile Arg Asn His Gln Ala Arg Gly Glu Glu Glu Lys Gly Leu
             20                  25                  30

Ala Thr Lys Asp Gln Gly Ile Cys Cys Glu Ile Val Ser Pro Glu Leu
         35                  40                  45

Ile Ile Lys Asp Leu Phe Leu Cys Val Trp Ile Cys Val Ser Val Gln
     50                  55                  60

His Leu Tyr Ala Val Leu Thr Glu Ala Arg Arg Ala Tyr Gln Ile Pro
 65                  70                  75                  80

Trp Thr Trp Arg Tyr Arg Gln Leu Leu Asp Pro Met Lys Val Leu Gly
                 85                  90                  95

Thr Lys Pro Arg Leu Ser Ala Lys Gln Pro Leu Ser Phe Leu Ser Phe
            100                 105                 110

Ile
```

<210> 3
<211> 2309
<212> DNA
<213> Mus musculus

<220>
<223> Sequence for TSG-5 coding and non-coding regions;
Hind III fragment

<220>
<221> unsure

<222> (187)..(188)
<223> w = a or t

<220>
<221> unsure
<222> (306)..(345)
<223> r = a or g

<220>
<221> unsure
<222> (367)
<223> b = c or g or t

<220>
<221> unsure
<222> (395)..(406)
<223> m = a or c

<220>
<221> unsure
<222> (512)
<223> v = a or c or g

<220>
<221> unsure
<222> (519)
<223> d = a or g or t

<220>
<221> unsure
<222> (1208)..(1217)
<223> y = c or t

<220>
<221> unsure
<222> (1290)
<223> n = a or c or g or t

<400> 3
aagctttata aaccttgaat caccctaagt agaatttatc tgcctactga caagcagtct 60
cagtgtggcc attcaccata ggtacttaca gggcttccaa aacccctgtt tgatgtctct 120
gcccacattg cccttgtgga ccaagttggc caatgaccca caagagcttg gagacccaca 180
tttctawwtg attctgattc atggtttaaa atttgaaatt aaactcaagt acttttacac 240
tctggtcaac ctaataaaat aaaaattgat ttaacaaaat tctccaaacc accactctga 300
aaaggrcaaa acattgttga gagaaagagg agaaaaccaa agtargtgaa aatatgacat 360
gcgtctbggg gttggaaatg tcatcctatt agcamaacct tccccmaatt aatctacaga 420
tttaagatat gttaaaaaat attgcaggat gccttttcga gctgatccta aaatgtacac 480
atacccaaaa taaacaaaac tatggaaata avagaaaadg tagaaaggat ctcctagttt 540
taaaacctac tttaaaccta tataaatatg acagtttaaa ggacccatca tttaatatat 600
gaggggctct ttcagtatgc cagtgtatct gagcaaaaag aaagaaactt atgagactgt 660
tctgcctact aaaattaagg tagctgctcc cacagtgaaa accttgattc accactccca 720
accagaaaag gtcattaagg attcaaagaa acgttgagac attgtttgaa tattgccact 780
actgaaatta gacaaagaat gacagggatt tgtctagaga ttctcaagtc atagaatact 840
atcactactc aaattatctt cttacacact tttaaataat atagaattat attattcttc 900
cctctgcccc ttcctccctc cagtccttct tatatacctc ctgccctagc tacttctcaa 960
aattataacc tcttctttat tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg 1020

tgtgtgtaaa taaacttcat tagtgttttt agtggttctt gaaagtatct gatttctggg 1080
gataaccact tggtatttga taatcaattt gggggctcac cccttaggga agactatttc 1140
tctggctctc agcctctgta agtttcctat ggttctttga atatgggaaa gacaccatga 1200
aatttttycc ctttccygtt agtgtgttac cttcagattt ggtttaggca gtcatactgt 1260
acatgtatct tgtgtgagac ataatacctn aactgcaatc taaatatgta ttttacctgc 1320
agataactat agctctcaac caatcattaa tcaaacttct cttgcaacaa atggggacca 1380
ttacagaaaa ctacagcctt gcaaaatgca gggaatgaat ggggagccca gtggctatta 1440
ccacaccctc aaaacaactc ctgtgccaaa ggatcagaaa tcatcaagca agaggggaag 1500
aagaaaaagg gttagcaacg aaggaccagg gaatctgctg tgagattgtg tctcctgaac 1560
tgattattaa agatttattt ttatgtgtat ggatatgtgt gtctgtgcag catttatatg 1620
cagtgcttac agaggctaga agagcatatc agatcccctg gacctggagg tacagacagt 1680
tattagaccc catgaaagtg ctaggaacca aacccaggct ctctgcaaag caaccactga 1740
gctttctctc ctttatttaa aaataaagat gatagataaa tcatctttat tttattctaa 1800
acacctaaat tgtgctgtag ggttttcatc tcagaatatt atctcataga ttttattgat 1860
tacaacccat tgtcaaaagt gtttctgatc tctggaatca gaatattccc ctattctttt 1920
tagtataatt tcccaaagcc tacataatgc tgagacttag ttttataagt gcaaaattgt 1980
tttgcctttt ctgcaatatg attcatttta attgccaata agaatcttca ttttttgatg 2040
acatatcaca ttcattctga tttacaagat ttgagaaaat gaagaagaaa tttgctgtct 2100
cattattctc tatctagaac caatgcaact cttccatgtt tctatacccc agaccaggga 2160
cataagcatc tctgcagcag cctcacatcc ccacttgacc acacgccata ttgcttctgt 2220
ctcctacact gaatcactta aaactaagtg aagcactccc actagctttg caaaaacaca 2280
tcatctaaac acactcttca gaaaagctt 2309

<210> 4
<211> 3862
<212> DNA
<213> Mus musculus

<220>
<223> TSG-5 genomic sequence

<220>
<221> unsure
<222> (509)
<223> n = a or c or g or t

<220>
<221> unsure
<222> (1073)..(1074)
<223> w = a or t

<220>
<221> unsure
<222> (1192)..(1231)
<223> r = a or g

<220>
<221> unsure
<222> (1253)
<223> b = c or g or t

<220>
<221> unsure
<222> (1281)..(1292)
<223> m = a or c

<220>
<221> unsure
<222> ()..)
<223> y = c or t

<220>
<221> unsure
<222> (2176)..(3862)
<223> n = a or c or g or t at positions 2176, 3577, 3678, 3774, 3790, 3862

<400> 4
tctagatgag gacatgaaaa tagagagcat ctaccccaga agggaaacaa tgatgctatg 60
ctactcccat taagcatgat ccacggaaag tcatcagggg agtgcagctt taaaattaat 120
aaaaattcta agtggattgt ataatgttgc cagttacaaa ttcaacatag gaaactcaat 180
gtgggaaaac actgctagtt aaagtcaaag cccaccatga cccgatagcc tctaggctta 240
gcacaatata ggagactccc atttgtggca ggtatctcct tatagagcct tgtccagggg 300
attcttttat tcttttctta ttgaatattt tctttattta catttcaaat gttatcccct 360
ttcctggttt ctccccagaa gcccacatcc ctccctctc ccctacttct attagggtgt 420
tcccctaccc atccacccac tcctgcttcc catcctggca ttctctacac tggggcatcg 480
agccttcata ggaccaaggg cctctcctnc cactgatgcc caacaagggc atcctctgct 540
catatgtagc tggagccatg ggcccctcca tatgtacact ttagtccctg gaagctctgt 600
ggggtctgat tggttgatat tattgttctt cctatggggt tgcaaaccct tcagctcctt 660
cagtccttta tttaactcct ccattgggga tccacacttg cctctacctg aggaatgtca 720
tgtagcctca attataggac caatttcatt tctcctggaa aaactcgtac aaataatact 780
taagactgtt gaaatgtttc cccatgctaa tgaggcattc caagtaccct aagaccccag 840
ccaatgacct ttgcccatcc cggatgtctc taccctcagt cctgcaaagc tttataaacc 900
ttgaatcacc ctaagtagaa tttatctgcc tactgacaag cagtctcagt gtggccattc 960
accataggta cttacagggc ttccaaaacc cctgtttgat gtctctgccc acattgccct 1020
tgtggaccaa gttggccaat gacccacaag agcttggaga cccacatttc tawwtgattc 1080
tgattcatgg tttaaaattt gaaattaaac tcaagtactt ttacactctg gtcaacctaa 1140
taaaataaaa attgatttaa caaaattctc caaaccacca ctctgaaaag grcaaaacat 1200
tgttgagaga aagaggagaa aaccaaagta rgtgaaaata tgacatgcgt ctbggggttg 1260
gaaatgtcat cctattagca maaccttccc cmaattaatc tacagattta agatatgtta 1320
aaaaatattg caggatgcct tttcgagctg atcctaaaat gtacacatac ccaaaataaa 1380
caaaactatg gaaataaaag aaaaagtaga aaggatctcc tagttttaaa acctacttta 1440
aacctatata aatatgacag tttaaaggac ccatcattta atatatgagg ggctctttca 1500
gtatgccagt gtatctgagc aaaaagaaag aaacttatga gactgttctg cctactaaaa 1560
ttaaggtagc tgctcccaca gtgaaaacct tgattcacca ctcccaacca gaaaaggtca 1620
ttaaggattc aaagaaacgt tgagacattg tttgaatatt gccactactg aaattagaca 1680
aagaatgaca gggatttgtc tagagattct caagtcatag aatactatca ctactcaaat 1740
tatcttctta cacactttta aataatatag aattatatta ttcttccctc tgccccttcc 1800
tccctccagt ccttcttata tacctcctgc cctagctact tctcaaaatt ataacctctt 1860
ctttattgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtaaataaa 1920
cttcattagt gtttttagtg gttcttgaaa gtatctgatt tctggggata accacttggt 1980
atttgataat caatttgggg gctcaccccc tagggaagac tatttctctg gctctcagcc 2040
tctgtaagtt tcctatggtt ctttgaatat gggaaagaca ccatgaaatt tttycccttt 2100
ccygttagtg tgttaccttc agatttggtt taggcagtca tactgtacat gtatcttgtg 2160
tgagacataa tacctnaact gcaatctaaa tatgtatttt acctgcagat aactatagct 2220
ctcaaccaat cattaatcaa acttctcttg caacaaatgg ggaccattac agaaaactac 2280
agccttgcaa aatgcaggga atgaatgggg agcccagtgg ctattaccac accctcaaaa 2340
caactcctgt gccaaaggat cagaaatcat caagcaagag gggaagaaga aaaagggtta 2400
gcaacgaagg accagggaat ctgctgtgag attgtgtctc ctgaactgat tattaaagat 2460
ttatttttat gtgtatggat atgtgtgtct gtgcagcatt tatatgcagt gcttacagag 2520
gctagaagag catatcagat cccctggacc tggaggtaca gacagttatt agaccccatg 2580
aaagtgctag gaaccaaacc caggctctct gcaaagcaac cactgagctt tctctccttt 2640

atttaaaaat aaagatgata gataaatcat ctttatttta ttctaaacac ctaaattgtg 2700
ctgtagggtt ttcatctcag aatattatct catagatttt attgattaca acccattgtc 2760
aaaagtgttt ctgatctctg gaatcagaat attcccctat tctttttagt ataatttccc 2820
aaagcctaca taatgctgag acttagtttt ataagtgcaa aattgttttg ccttttctgc 2880
aatatgattc attttaattg ccaataagaa tcttcatttt ttgatgacat atcacattca 2940
ttctgattta caagatttga gaaaatgaag aagaaatttg ctgtctcatt attctctatc 3000
tagaaccaat gcaactcttc catgtttcta taccccagac cagggacata agcatctctg 3060
cagcagcctc acatccccac ttgaccacac gccatattgc ttctgtctcc tacactgaat 3120
cacttaaaac taagtgaagc actcccacta gctttgcaaa aacacatcat ctaaacacac 3180
tcttcagaaa agcttcaagt gaggatgtcc acacataagg catatgtaga aactgtatta 3240
tttgctttgt tttgctatga gaataaaaac tactctaaaa attaatagta tttatacaat 3300
tatcatcaaa actctccatc ctgatgccac tgcactgcac tatccacaaa taagccccag 3360
ggtataatgg aggtgcctac ttcttctgta atgtctttcc actcctttat cctttgctc 3420
ctcagaagtg cagatgttca gaccactcta ggccatagcc ctcccgctga agtctgcacc 3480
catctgtcac tcagacagac agcctattca ttctgtcggg accagttcat cctcccccat 3540
tccagtgatg cctagctgac tctccagcaa aaccagngtc ttatggtcta attatatgtt 3600
ttgcatattt aatttttccc aggacttctt tgaaagccaa cctgtattta aaagagtatt 3660
ttccaacttc caaagttntt taaaggtata ccagctatta caagacaaat agaaaacatc 3720
accttcagct cctcttggcc agtgttttga tatgctttag gaaacaaagg gcangtccct 3780
tcaagcatgn gaatgtgctc taatctttaa ggcgacaccc cagcccagaa acatggttca 3840
ctttggaatn gagtctattt ga 3862

<210> 5
<211> 350
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
sequence with 5 nucleotide changes which
hybridizes to TSG-5

<400> 5
gggaatgaac ggggagccca gtggctatta ccacaccctc aaaaccactc ctgtgccaaa 60
ggatcagaaa tcatcaagca agaggggaag aagaaaaagg gttagcaacg aaggaccagg 120
gaatctgctg tgagactgtg tctcctgaac tgattattaa agatttattt ttatgtgtat 180
ggatatgtgt gtatgtgcag catttatatg cagtgcttac agaggctaga agagcatatc 240
agatcccctg gacctggagg tacagacagt tataagaccc catgaaagtg ctaggaacca 300
aacccaggct ctctgcaaag caaccactga gctttctctc ctttatttaa 350

<210> 6
<211> 350
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
sequence with 3 nucleotide changes which
hybridizes to TSG-5

<400> 6
gggaatgaac ggggagccca gtggctatta ccacaccctc aaaaccactc ctgtgccaaa 60
ggatcagaaa tcatcaagca agaggggaag aagaaaaagg gttagcaacg aaggaccagg 120
gaatctgctg tgagactgtg tctcctgaac tgattattaa agatttattt ttatgtgtat 180

ggatatgtgt gtctgtgcag catttatatg cagtgcttac agaggctaga agagcatatc 240
agatcccctg gacctggagg tacagacagt tattagaccc catgaaagtg ctaggaacca 300
aacccaggct ctctgcaaag caaccactga gctttctctc ctttatttaa 350

<210> 7
<211> 253
<212> DNA
<213> Mus musculus

<220>
<223> GenBank Accession No. AF004564, clone no. 059A14+

<400> 7
ccaatcatta atcaaacttc tcttgcaaca aatggggacc attacagaaa actacagcct 60
tgcaaaatgc agggaatgaa tggggagccc agtggctatt accacaccct caaaacaact 120
cctgtgccaa aggatcagaa atcatcaagc aagaggggaa gaagaaaaag ggttagcaac 180
gaaggaccag ggaatctgct gtgagattgt gtctcctgaa ctgattatta aagatttatt 240
tttatgtgta tgc 253

<210> 8
<211> 1238
<212> DNA
<213> Mus musculus

<220>
<223> Promoter region of TSG-5 gene

<220>
<221> variation
<222> (40)..(41)
<223> w = a or t

<220>
<221> variation
<222> (159)..(198)
<223> r = a or g

<220>
<221> variation
<222> (220)
<223> b = c or g or t

<220>
<221> variation
<222> (248)..(259)
<223> m = a or c

<220>
<221> variation
<222> (365)
<223> v = a or c or t

<220>
<221> variation

<222> (372)
<223> d = a or g or t

<220>
<221> variation
<222> (1061)..(1070)
<223> y = c or t

<220>
<221> variation
<222> (1143)
<223> n = a or c or g or t

<400> 8
ggccaatgac ccacaagagc ttggagaccc acatttctaw wtgattctga ttcatggttt 60
aaaatttgaa attaaactca agtactttta cactctggtc aacctaataa aataaaaatt 120
gatttaacaa aattctccaa accaccactc tgaaaaggrc aaaacattgt tgagagaaag 180
aggagaaaac caaagtargt gaaaatatga catgcgtctb ggggttggaa atgtcatcct 240
attagcamaa ccttccccma attaatctac agatttaaga tatgttaaaa aatattgcag 300
gatgcctttt cgagctgatc ctaaaatgta cacataccca aaataaacaa aactatggaa 360
ataavagaaa adgtagaaag gatctcctag ttttaaaacc tactttaaac ctatataaat 420
atgacagttt aaaggaccca tcatttaata tatgaggggc tctttcagta tgccagtgta 480
tctgagcaaa aagaaagaaa cttatgagac tgttctgcct actaaaatta aggtagctgc 540
tcccacagtg aaaaccttga ttcaccactc ccaaccagaa aaggtcatta aggattcaaa 600
gaaacgttga gacattgttt gaatattgcc actactgaaa ttagacaaag aatgacaggg 660
atttgtctag agattctcaa gtcatagaat actatcacta ctcaaattat cttcttacac 720
acttttaaat aatatagaat tatattattc ttccctctgc cccttcctcc ctccagtcct 780
tcttatatac ctcctgccct agctacttct caaaattata acctcttctt tattgtgtgt 840
gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt gtgtgtgtgt aaataaactt cattagtgtt 900
tttagtggtt cttgaaagta tctgatttct ggggataacc acttggtatt tgataatcaa 960
tttgggggct caccccttag ggaagactat ttctctggct ctcagcctct gtaagtttcc 1020
tatggttctt tgaatatggg aaagacacca tgaaattttt ycccttccy gttagtgtgt 1080
taccttcaga tttggtttag gcagtcatac tgtacatgta tcttgtgtga gacataatac 1140
ctnaactgca atctaaatat gtattttacc tgcagataac tatagctctc aaccaatcat 1200
taatcaaact tctcttgcaa caaatgggga ccattaca 1238

<210> 9
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
Antibody-inducing peptide from TSG-5

<400> 9
Arg Asn His Gln Ala Arg Gly Glu Glu Glu Lys Gly Leu Ala Thr Lys
1 5 10 15

Asp Gln Gly Ile Cys
20

<210> 10
<211> 13

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer for reverse transcription

<400> 10
tttttttttt tvn 13

<210> 11
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Forward PCR primer

<400> 11
caaaatgcag ggaatgaatg 20

<210> 12
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Reverse PCR primer

<400> 12
agagagcctg ggtttggtt 19

<210> 13
<211> 333
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (11)..(280)
<223> TSG-5 cDNA

<400> 13
atcagctctg tgc ttc ctg acc tgc ttg agt tcc agt cct gac ttc ctt 49
Cys Phe Leu Thr Cys Leu Ser Ser Ser Pro Asp Phe Leu
1 5 10

ggt gat gaa cgc aat aac tat agc tct caa cca atc att aat caa act 97
Gly Asp Glu Arg Asn Asn Tyr Ser Ser Gln Pro Ile Ile Asn Gln Thr
15 20 25

```
tct ctt gca aca aat gga gac cat tca cga aaa cta cag cct tgc aaa   145
Ser Leu Ala Thr Asn Gly Asp His Ser Arg Lys Leu Gln Pro Cys Lys
 30                  35                  40                  45

atg cag gga atg aat ggg gag ccc agt ggc tat tac cac acc ctc aaa   193
Met Gln Gly Met Asn Gly Glu Pro Ser Gly Tyr Tyr His Thr Leu Lys
                 50                  55                  60

aca act cct gtg cca aag gat cag aaa tca tca agc aag agg gga aga   241
Thr Thr Pro Val Pro Lys Asp Gln Lys Ser Ser Ser Lys Arg Gly Arg
             65                  70                  75

aga aaa agg gtt agc aac gaa gga cca ggg aat ctg ctg tgagattgtg    290
Arg Lys Arg Val Ser Asn Glu Gly Pro Gly Asn Leu Leu
         80                  85                  90

tctcctgaat tgattattaa agatttattt ttatgtgtat gga                  333
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<210> 14
<211> 90
<212> PRT
<213> Mus musculus

<400> 14

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Cys Phe Leu Thr Cys Leu Ser Ser Ser Pro Asp Phe Leu Gly Asp Glu
  1               5                  10                  15

Arg Asn Asn Tyr Ser Ser Gln Pro Ile Ile Asn Gln Thr Ser Leu Ala
             20                  25                  30

Thr Asn Gly Asp His Ser Arg Lys Leu Gln Pro Cys Lys Met Gln Gly
         35                  40                  45

Met Asn Gly Glu Pro Ser Gly Tyr Tyr His Thr Leu Lys Thr Thr Pro
     50                  55                  60

Val Pro Lys Asp Gln Lys Ser Ser Ser Lys Arg Gly Arg Arg Lys Arg
 65                  70                  75                  80

Val Ser Asn Glu Gly Pro Gly Asn Leu Leu
                 85                  90
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<210> 15
<211> 330
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (2)..(277)
<223> TSG-5 cDNA

<400> 15

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t gac ctc tgc atc agc tcc tgc ttc ctg acc tgc ttg agt tcc agt cct 49
  Asp Leu Cys Ile Ser Ser Cys Phe Leu Thr Cys Leu Ser Ser Ser Pro
    1               5                  10                  15

gac ttc ctt gat aac tat agc tct caa cca atc att aat caa act tct   97
Asp Phe Leu Asp Asn Tyr Ser Ser Gln Pro Ile Ile Asn Gln Thr Ser
             20                  25                  30

ctt gca aca aat gga gac cat tca cga aaa cta cag cct tgc aaa atg   145
Leu Ala Thr Asn Gly Asp His Ser Arg Lys Leu Gln Pro Cys Lys Met
         35                  40                  45

cag gga atg aat ggg gag ccc agt ggc tat tac cac acc ctc aaa aca   193
Gln Gly Met Asn Gly Glu Pro Ser Gly Tyr Tyr His Thr Leu Lys Thr
     50                  55                  60

act cct gtg cca aag gat cag aaa tca tca agc aag agg gga aga aga   241
Thr Pro Val Pro Lys Asp Gln Lys Ser Ser Ser Lys Arg Gly Arg Arg
 65                  70                  75                  80

aaa agg gtt agc aac gaa gga cca ggg aat ctg ctg tgagattgtg        287
Lys Arg Val Ser Asn Glu Gly Pro Gly Asn Leu Leu
                 85                  90

tctcctgaat tgattattaa agatttattt ttatgtgtat gga                  330
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<210> 16
<211> 92
<212> PRT
<213> Mus musculus

<400> 16

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  1               5                  10                  15

Asp Phe Leu Asp Asn Tyr Ser Ser Gln Pro Ile Ile Asn Gln Thr Ser
             20                  25                  30

Leu Ala Thr Asn Gly Asp His Ser Arg Lys Leu Gln Pro Cys Lys Met
         35                  40                  45

Gln Gly Met Asn Gly Glu Pro Ser Gly Tyr Tyr His Thr Leu Lys Thr
     50                  55                  60

Thr Pro Val Pro Lys Asp Gln Lys Ser Ser Ser Lys Arg Gly Arg Arg
 65                  70                  75                  80

Lys Arg Val Ser Asn Glu Gly Pro Gly Asn Leu Leu
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<210> 17
<211> 98
<212> PRT

<213> Homo sapiens

<220>
<223> Amino acid sequence of MIP3-beta

<400> 17

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Met Ala Leu Leu Leu Ala Leu Ser Leu Leu Val Leu Trp Thr Ser Pro
  1               5                  10                  15

Ala Pro Thr Leu Ser Gly Thr Asn Asp Ala Glu Asp Cys Cys Leu Ser
             20                  25                  30

Val Thr Gln Lys Pro Ile Pro Gly Tyr Ile Val Arg Asn Phe His Tyr
         35                  40                  45

Leu Leu Ile Lys Asp Gly Cys Arg Val Pro Ala Val Val Phe Thr Thr
     50                  55                  60

Leu Arg Gly Arg Gln Leu Cys Ala Pro Pro Asp Gln Pro Trp Val Glu
 65                  70                  75                  80

Arg Ile Ile Gln Arg Leu Gln Arg Thr Ser Ala Lys Met Lys Arg Arg
                 85                  90                  95

Ser Ser
```

INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/US 99/22711

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/19 C12N1/21 C07K14/52 G01N33/68 C12Q1/68
C07K16/24 A61K51/00 A61K38/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	E.G. PIRES ET AL: "Mus musculus TNF-responsive gene sequence" EMBL DABASE ENTRY AF042095, ACCESSION NUMBER AF042095 , 23 January 1998 (1998-01-23), XP002128359 cited in the application abstract ---	1-8,11, 12,14, 15,30
X	E. G. PIRES ET AL: "Mus musculus clone 059A14+ TNF alpha induced mRNA sequence" EMBL DATABASE ENTRY MMAF4564, ACCESSION NMUBER AF04564,5 July 1997 (1997-07-05), XP002128360 abstract --- -/--	1-8,11, 12,14, 15,30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 January 2000	03/02/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter: ınal Application No
PCT/US 99/22711

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92 12175 A (UNIV NEW YORK) 23 July 1992 (1992-07-23) page 66 -page 67; examples IV,VIII,X,XIV; table 3 page 86 -page 89; table 6 claims	1-36
A	WO 94 07542 A (MALLINCKRODT MEDICAL INC ;UNIV MICHIGAN (US)) 14 April 1994 (1994-04-14) the whole document & US 5 605 671 A cited in the application	24-29
P,X	Q. WANG ET AL: "Mus musculus PAC clone 657p21" EMBL DATABASE ENTRY AC005743, ACCESSION NUMBER AC005743, 2 October 1998 (1998-10-02), XP002128361 abstract & UNPUBLISHED,	1-8,11, 12,14,15

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/22711

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
See FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims 30,32 all partially and 37-39 completely
See FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 30 and 32 (as far as they concern an in vivo method) are directed to a method of treatment of the human/animal body (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound. And although claims 24-29 are directed to a diagnostic method practised on the human/animal body (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 30, 32 all partially and 37-39 completely

Present claims 30 and 32 relate to a method referring to an agent defined by reference to a desirable characteristic or property, namely " which modulates the expression of a nucleic acid encoding the protein" or " which modulates at least one activity of the protein"

The claims cover all agents having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such agents. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the agent by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for claims 30 and 32 for those agents which appear to be clear, supported and disclosed, namely for those agents identified in example 4 page 40 figures 1 and 6: TNF-alpha, IFN-gamma and LPS.

Claims 37-39 refer to a method relating to an agonist/antagonist of the polypeptides without giving a true technical characterization. Moreover no such compounds are defined in the application. In consequence, the scope of said claimsis ambiguous and vague, and their subject matter is not sufficiently disclosed and supported.
No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

information on patent family members

Internal Application No
PCT/US 99/22711

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9212175 A	23-07-1992	AT 185573 T	15-10-1999
		AU 1228892 A	17-08-1992
		DE 69230136 D	18-11-1999
		EP 0567575 A	03-11-1993
		JP 6504912 T	09-06-1994
		US 5386013 A	31-01-1995
		US 5846763 A	08-12-1998
WO 9407542 A	14-04-1994	US 5413778 A	09-05-1995
		AT 150977 T	15-04-1997
		AU 674039 B	05-12-1996
		AU 5403294 A	26-04-1994
		CA 2145983 A	14-04-1994
		DE 69309479 D	07-05-1997
		EP 0662843 A	19-07-1995
		JP 8502075 T	05-03-1996
		US 5605671 A	25-02-1997